

EFFECT OF PROCESSING PARAMETERS ON THE RHEOLOGICAL AND
PHYSICO-CHEMICAL PROPERTIES OF VARIETAL APPLE SAUCE

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EFFECT OF PROCESSING PARAMETERS ON THE RHEOLOGICAL AND PHYSICO-CHEMICAL PROPERTIES OF VARIETAL APPLESAUCE

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Applesauce is made from a blend of varieties and processed year round due to the availability of apples from cold storage and controlled atmosphere storage, factors that affect applesauce quality. Applesauce attributes are also affected by processing operations; thus, understanding how these factors influence the final product will help to improve applesauce quality. Our goals were to assess the effect of processing parameters on rheological, physical and chemical properties of applesauce, considering variety and ripening effects, and to optimize processing conditions to improve sauce quality. We investigated the following factors: hot and cold break process; extractor rotational speed; addition of diced apples to extractor sauce output; addition of exogenous pectin methyl esterase (PME) and calcium; and activation of endogenous PME. Samples were analyzed for rheological properties (consistency index, yield stress, USDA consistency values) and physico-chemical properties (particle size distribution, pH, soluble solids, pectin content, pectin degree of methoxylation (DM)). Results were evaluated by ANOVA and Tukey's HSD test ($p \leq 0.05$).

Best sauce quality (thicker sauce with low syneresis) was achieved when applesauce had a mean particle size $\leq 700 \mu\text{m}$, particle size distribution span ≥ 1.75 ,

total soluble pectin $\geq 0.25\%$, and pectin DM $\leq 60\%$. Ripening or processing conditions could modify these parameters to meet targeted applesauce rheology. Processing effects differed with variety and ripening stage. Overall, higher rotational extractor speed and addition of $\geq 50\%$ diced apples to extractor sauce output prior to final pulping, resulted in increased sauce viscosity and decreased syneresis, due to an increase in total soluble pectin, a lower mean particle diameter and a wider particle size distribution. Exogenous PME addition caused a decrease in pectin DM, leading to higher sauce viscosity and less syneresis. When PME and Ca^{2+} were added, viscosity was highest but resulted in higher syneresis. Activation of endogenous PME at 55-60°C for 10 min lowered the pectin DM from 83% to 60-70% but no change on applesauce rheology was observed.

By understanding how sauce physico-chemical and rheological properties are affected by variety, ripening and processing parameters, it is possible to optimize all relevant conditions to achieve better consistency and quality.

BIOGRAPHICAL SKETCH

The author was born and raised in Bangkok, Thailand. She obtained her Bachelor of Science degree in Food Technology with 1st class honors from Chulalongkorn University, Bangkok, Thailand. After her undergraduate degree, she received a Fulbright Scholarship and Queen Sirikit, her Majesty the Queen Scholarship to pursue her graduate study. She started her Ph.D. program in Food Science and Technology at Cornell University under the supervision of Dr. Olga I. Padilla-Zakour. Her research was conducted on the area of food processing, focusing on applesauce. During her graduate studies, she was active in many extra-curriculum activities such as being a student representative (Geneva campus) for Western New York Institute of Food Technologists, a vice-president in SAGES (Student Association of the Geneva Experimental Station), and a product development team member. After completing her Ph.D., she will work in the food industry in her country in the area of research and development.

To my beloved family:
Dad, Mom, Fon and Boy

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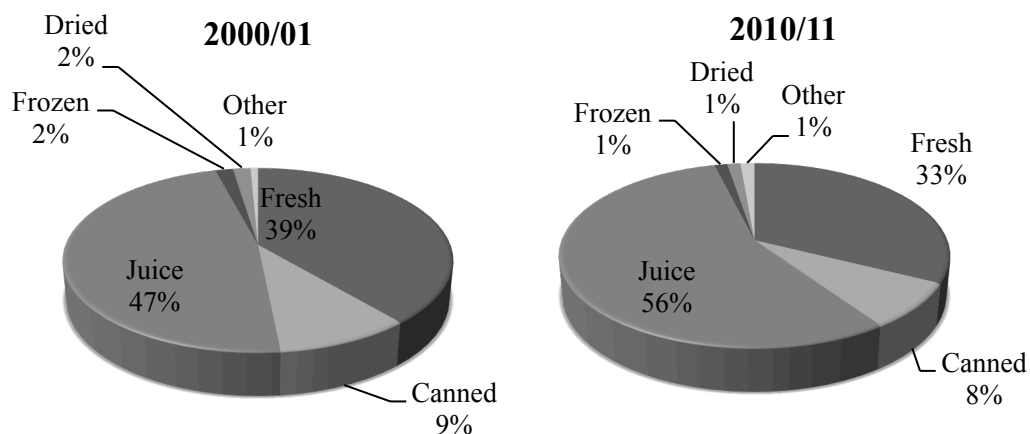
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CHAPTER 1

INTRODUCTION

The United States is the second largest apple producing country in the world after China. According to the USDA (2012), in 2010 33.3 million tons of apples were grown in China accounting for 48% of the total world production, while 4.2 million tons were grown in the U.S., representing 6% of the total world production.

In the U.S., apples are the second most consumed fruit (fresh and processed uses combined) following oranges (US Apple 2011). The vast majority of apples and apple products are consumed at home. In 2010, average U.S. per person consumption of all forms of apples was about 22 kg, representing a 10% increase from the 2000 average at 20 kg. The distribution of consumption per type of product is presented in Figure 1.1, showing an increase in the processed apple products contribution to apple intake over the last 10 years



Source: USDA Economics, Statistics and Market Information System, US apple statistics, 2012

Figure 1.1 U.S. per person consumption of apples in 2001/01 and 2010/11, by product

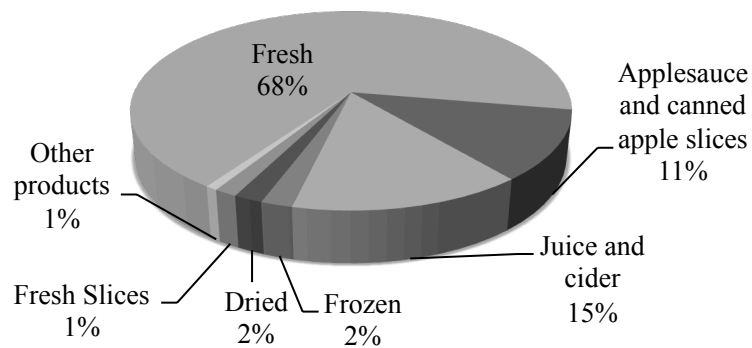
U.S. Apple Production and Utilization

Over the last 10 years, apple production and utilization in the U.S. has remained fairly constant, with year-to-year variations due to growing conditions affecting crop load and harvest. In 2010, apples utilized production was 4.18 million tons, valued at \$2.22 billion, with apples for fresh consumption representing 68% while 32% were processed, as shown in Table 1.1 (USDA 2012). Of the processed apples, the top products are apple juice and cider, followed by canned products including applesauce and canned apple slices, frozen, dried, and fresh slices, as depicted in Figure 1.2. Tables 1.2 and 1.3 indicate the importance of the canned apples category as they represent a higher economic value than juice and cider even though production volume is smaller.

Table 1.1 Apples: production, utilization, and value in the United States, 2001-2010

Year	Production (million tons)		Utilization (million tons)		Values of Utilized Production (billion dollars)		
	Total	Utilized	Fresh	Processed	Fresh	Processed	All
2001	4.27	4.18	2.48	1.70	1.25	0.20	1.45
2002	3.87	3.80	2.43	1.36	1.39	0.20	1.58
2003	3.98	3.94	2.47	1.47	1.42	0.21	1.63
2004	4.72	4.69	3.00	1.68	1.21	0.20	1.41
2005	4.38	4.34	2.77	1.57	1.47	0.18	1.66
2006	4.46	4.41	2.86	1.55	1.99	0.22	2.21
2007	4.12	4.10	2.76	1.35	2.33	0.28	2.61
2008	4.37	4.33	2.85	1.48	1.89	0.32	2.21
2009	4.40	4.29	2.86	1.42	1.98	0.21	2.19
2010	4.22	4.18	2.86	1.33	1.95	0.27	2.22

Source: USDA Economics, Statistics and Market Information System, US apple statistics, 2012



Source: USDA Economics, Statistics and Market Information System, US apple statistics, 2012

Figure 1.2 Utilization of 2010 U.S. apple crop

Table 1.2 Apples: Processing utilization in the United States (Volume)

	Apple Utilization (thousands tons)					
	2006	2007	2008	2009	2010	2011
Canned*	529.5	495.0	568.5	525.4	473.2	509.7
Juice and cider	703.2	570.3	611.7	630.2	606.0	548.1
Frozen	123.3	116.9	95.8	107.1	82.1	86.4
Dried	114.7	92.4	96.5	73.1	75.1	83.2
Fresh Slices	53.6	44.6	58.0	63.0	59.6	108.1
Others	27.9	27.2	50.8	25.1	31.3	32.2

Source: USDA, National Agricultural Statistics Service, *Noncitrus Fruits and Nuts Summary*, various years

*Canned includes applesauce and canned apple sliced

Table 1.3 Apples: Processing utilization in the United States (Value)

	Apple Utilization (million \$)					
	2006	2007	2008	2009	2010	2011
Canned*	92.2	100.4	150.4	93.2	106.4	117.2
Juice and cider	79.1	112.5	94.4	63.8	98.2	108.5
Frozen	22.7	30.7	26.4	18.2	19.1	21.3
Dried	7.8	18.5	8.0	4.3	11.8	17.8
Fresh Slices	14.2	14.1	31.1	22.6	25.2	38.6
Others	5.0	5.7	12.8	4.6	5.7	6.5

Source: Source: USDA, National Agricultural Statistics Service, *Noncitrus Fruits and Nuts Summary*, various years

*Canned includes applesauce and canned apple sliced

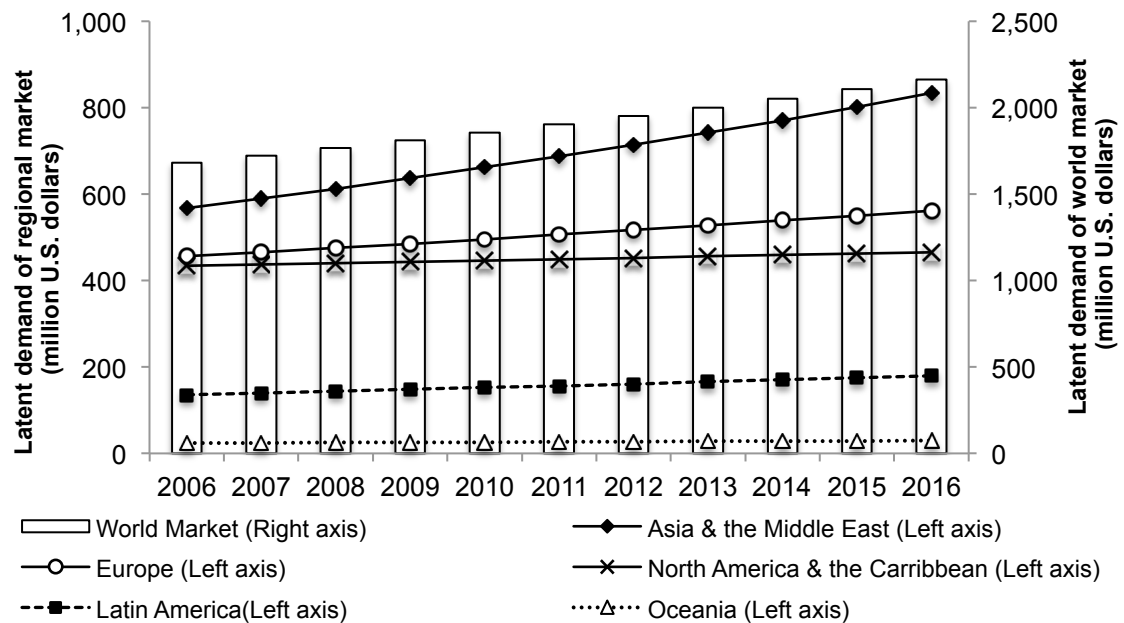
Apples are grown throughout the U.S. with the largest producing state being Washington, which accounts for 60% of the total utilized production. Other primary apple producers are New York (14%), Michigan (6%), Pennsylvania (5%), and California (3%). From 2000 to 2010, Washington was the top state producer of juice and cider products, representing about 40-60 percent of the nation's total production. However, New York is the top state for canned apple products, processing about 20-33 percent of all canned apples (USDA 2012).

Applesauce Market

Several factors contribute to increased apple and apple products consumption including the availability of new varieties, the lower prices of fruits and vegetables, the increase awareness of nutritional benefits of fruits (USDA 2010), and the recommendation from the 2010 Dietary Guidelines for Americans to consume two cups of fruit and two and a half cups of vegetables per day for a reference 2,000-calorie intake. Even though canned fruit consumption fell 7% from 2005 to 2008 due to the consumers' preference to fresh fruit or to other types of processed fruits, canned applesauce consumption increased 5%. Canned apples and applesauce were the most popular canned fruit in 2005 and 2008, accounting for 30% of total canned fruits consumption (USDA 2008, 2010). Additionally, a wave of new products' packaging could stimulate the growth of canned fruit market such as "cupped" fruit in single-serve plastic containers, or blended fruit or puree in pouches. These packaging options offer consumers an easy, ready-to-grab and go-snack product (Mintel 2009, 2013). Canned fruit suppliers have also kept pace with consumers' changing dietary

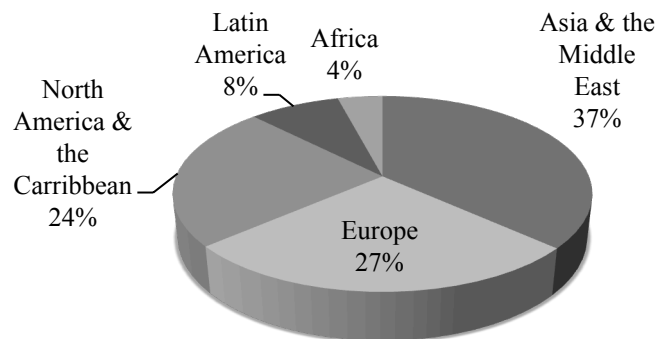
preferences to introduce no/low-sugar, all-natural, and value-added products and claims. For example, three recent applesauce products were fortified with antioxidants, calcium or fiber (Mintel 2009). The variety of packaging presentations and formulations provide an opportunity to meet consumer demands and to increase applesauce market share.

Worldwide, Parker (2010) estimated a 13% increase in the latent demand, or potential industry earnings, for canned applesauce by 2016, with Asia & the Middle East showing the highest increase (21%) followed by Latin America (15%) and Oceania (12%). Europe and North America, which ranked second and third in global demand, are expected to expand only 11 % and 4 %, respectively, as can be seen in Figure 1.3. The latent demand was calculated based on information available from 2006 to 2010 and estimated from 2006 to 2016. In essence, if applesauce companies target the top 3 regions—Asia & the Middle East, Europe and North America, they could cover 86% of the global latent demand for canned applesauce. China showed the greatest increase in demand of canned applesauce, while it is considered flat for the United States. The latent demand of canned applesauce was estimated to be \$1.9 billion in 2011. The distribution of the world market potential by regions is shown in Figure 1.4. The top country for canned applesauce demand is still the United States, accounting for 20% of the global demand, followed by China, Japan, India, and Germany.



Source: Parker, 2010

Figure 1.3 World and regional market for canned applesauce: 2006 - 2016



Source: Parker, 2010

Figure 1.4 Worldwide market potential for canned applesauce (based on 2011 total latent demand: \$1.9 billion)

Applesauce Processing and Quality

According to the Codex Alimentarius (Codex Stan 17-1981), applesauce is defined as “a comminuted or chopped product prepared from clean, washed and

possibly peeled apples of *Malus domestica* Borkhausen and processed by heat appropriately, prior to being placed in a hermetically sealed container to prevent spoilage”. The most common use of applesauce is as snacks or a side dish accompanied by pork products like ham or bacon. It is also presented as a healthy and convenient alternative to candy and chocolate bars, often eaten between meals, especially, in the case of children (Colin-Henrion and others 2009).

One of the most important characteristics of applesauce is its consistency, the flow behavior of the product and the degree of separation of free liquid (Schijvens and others 1998). Sila and others (2009) and Lopez-Sanchez and others (2011) reported that the rheological properties of plant-food dispersions are related to parameters such as particle size, morphology, pulp content, and pectic substances. Applesauce production has changed from a hot break process, where apple slices are cooked prior to pulping, to a more efficient cold break process, resulting in higher variability in consistency depending on apple variety and ripeness, and in some cases, leading to thinner sauce and excess free liquid. Additionally, consumer complaints are often related to excessive free liquid or thin sauce, which can also cause problems at the filling step. Applesauce with substandard consistency can overflow the primary package prior to capping or sealing, resulting in considerable financial losses for the industry.

Many studies on applesauce have been conducted with the hot break process, but primarily focused on the mechanical properties of applesauce in relation to the processing parameters utilized such as screen opening size, finisher rotational speed, and blanching conditions. As applesauce processors have changed from the hot to cold

break due to the utilization of a turbo extractor that can extract puree from fruits and vegetables at room temperature (US patent No. 0269564 2007), it is very important to understand how processing conditions, apple variety, and ripening affect the quality of the final sauce, as process efficiencies and final product quality will have an effect on product revenue and consumers' acceptability.

This study aimed to obtain a better understanding of the difference between hot and cold break process on applesauce quality and the effects of relevant processing conditions (extractor rotational speed, addition of diced apples to extractor sauce output, pectin methyl esterase activity) on the rheological and physico-chemical properties of cold break applesauce, taking apple variety and ripening stage into consideration. Additionally, we established correlations between applesauce rheological properties and physical and chemical attributes, thus facilitating the development of optimal processing methods that result in quality improvements and consistent products.

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CHAPTER 2

LITERATURE REVIEW

Applesauce Processing

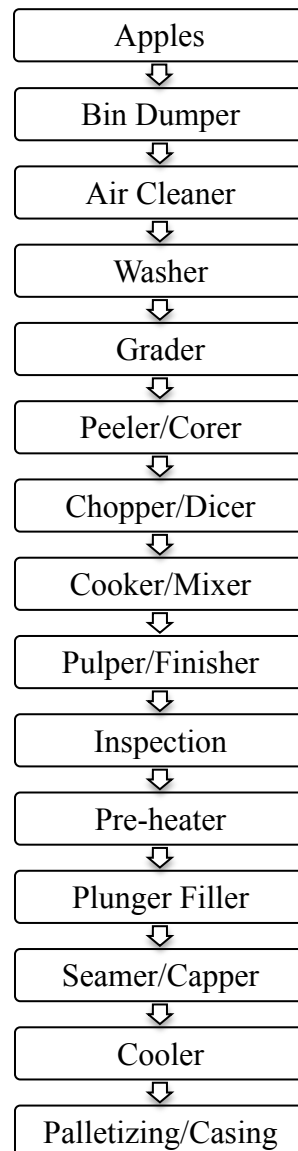
In the United States, applesauce is processed year round from apples harvested from August to November (Calvin and Martin 2010) and kept in cold storage (CS) – 1-4 °C and 95-98% relative humidity (RH) for up to 6 to 9 months – or controlled atmosphere storage (CA) – 1-3% O₂ and 1-4% CO₂ at 1-4 °C and 95-98% RH – for up to 9 to 12 months, respectively, depending on variety and storage condition (Louis and Massey 1989). Applesauce manufacturers typically use at least two or three varieties to meet applesauce quality specifications. Most apple varieties can be used for processing applesauce but only a few are considered ideal (Wiley and Binkley 1989). Quality attributes in raw apples that produce high quality finished product (thicker sauce and less syneresis) should include 1) good flavor with sufficient sugar and acid solids, 2) variability in particle size, and 3) high water-holding capacity of the apple tissue to pick up the condensate during the cooking operation (Labelle 1981; Wiley and Binkley 1989).

In accordance with the 21 CFR 145.110, applesauce is the food prepared from comminuted or chopped apples which may or may not be peeled and cored, and which may have added thereto one or more of the optional ingredients: water, apple juice, salt, any organic acid added for the purpose of acidification, nutritive carbohydrate sweeteners, spices and natural and artificial flavoring. Processing operations for applesauce are summarized in Figure 2.1. The processing of applesauce generally starts at a receiving point where the apples are inspected and unusable apples

discarded. Apples are then peeled, cored, diced or chopped and fed into a stainless steel type cooker, either live steam injected or steam jacketed. Sugar, either liquid blend or dry, and other desired ingredients are added to the sauce just before cooking. Cooking at 93-98°C is used to inactivate the polyphenoloxidase to prevent browning and to soften the apples before further processing. Cooking time varies with variety, depending on the firmness of the specific variety being used. For the hot break process, the cooked, softened apples are passed through a finisher and run through screens to remove the skins and seeds and obtain a smooth or grainy texture, depending on the size of the screen. The product is then thermally processed at 90°C, hot filled into plastic cups, glass jars or metal containers, and hermetically sealed. The containers are inverted for 1 to 2 min prior to cooling to insure sterilization of the lids or caps and then cooled down to room temperature.

A newer method for sauce production uses a cold break process by utilizing a turbo extractor to obtain juice or puree from food products at room temperature (US Patent 0269564 2007). Due to the advantages offered, many applesauce processors have changed from hot to cold break processing lines. The main advantages of cold break process are lighter color and better flavor as the product is exposed to less heat and cooked taste is minimized. In the hot break process, apples are chopped and cooked before feeding them to the pulper or finisher. With a turbo extractor, whole apples can be processed into sauce at room temperature following two operations that happen consecutively within sections of the machine: softening and extracting. The softening section has a rotor with blades to chop the whole apples and a cylindrical body (stator) to soften chopped apples by pushing the products by centrifugal force

against the protrusions of the stator. Then the softened chopped apples are pushed radially and continuously against the screen by centrifugal forces, thus extracting the mostly liquid and pulp components of the fruit that make the sauce. The solid parts that do not pass through the screen, comprising skins and seeds, are pushed and conveyed to the waste section.



Source: Wiley and Binkley, 1989

Figure 2.1 Unit operations in applesauce processing

Rheological Properties of Applesauce

Rheology is the study of deformation and flow of foods under well-defined conditions. The relevance of food rheology has been summarized into four categories, namely plant design, quality control, sensory attributes, and research and development of food structure (Escher 1983; Bourne 1992; Steffe 1992). Rheology is also relevant to the sensory and consumer perception of products due to its relation to density, viscosity, surface tension, and other physical properties (Matz 1962; McKenna 1990).

Plant-based food dispersions are complex materials made of insoluble solids (pulp) and liquid media (serum) (Tangerlertpaibul and Rao 1987). The plant food particles are hydrated and in equilibrium with the serum, which is an aqueous solution of sugars, organic acids, salts, and pectic substances, depending on factors such as variety and extent of ripening. The differences in characteristics of plant food dispersions can lead to significant interparticle forces, resulting in different flow and rheological characteristics in food products (Rao 2007).

A flow model may be considered to be a mathematical equation that can describe rheological data, such as shear rate versus shear stress.

Power Law Model

Shear stress-shear rate plots of many fluids become linear when plotted on double logarithmic coordinates and the power law model describes the data of shear-thinning and shear thickening fluids:

$$\sigma = K\dot{\gamma}^n \quad \text{Equation 2.1}$$

where σ is the shear stress, K is the consistency coefficient, in Pa, $\dot{\gamma}^n$ is the shear rate stress at a shear rate of 1.0 s^{-1} , and the exponent n , the flow behavior index, is the

dimensionless number that reflects the closeness to Newtonian flow.

Casson Model

The Casson model is a structure-based model and has been used to characterize a number of food dispersions:

$$\sigma^{0.5} = K_{0C} + K_C(\dot{\gamma})^{0.5} \quad \text{Equation 2.2}$$

For a food whose flow behavior follows the Casson model, a straight-line results when the square root of shear rate, $(\dot{\gamma})^{0.5}$, is plotted against the square root of shear stress, $(\sigma^{0.5})$, with slope K_C and intercept K_{0C} . The Casson Yield stress is calculated as the square of the intercept, $\sigma_{0C} = (K_{0C})^2$ and the Casson plastic viscosity as the square of the slope, $\eta_{Ca} = (K_C)^2$.

In commercial applications, applesauce quality is defined by the U.S. Standards for Grade of Canned Applesauce (USDA 2005) in terms of five attributes: color, consistency, absence of defects, finish and flavor. Consistency refers to the “the flow characteristics of the product and the degree of separation of free liquid”, while finish means “the texture and tenderness of apple particles”. Both attributes are primarily due to rheological properties and size of the apple tissue particles in the sauce (Nogueira and others 1985). Rao and others (1986) stated that all applesauce samples were pseudoplastic and correlated well with the power law’s and Casson’s models. Previous studies have focused on the rheological properties of applesauce in relation to the processing parameters applied, and characterized their flow properties (consistency index, flow behavior, yield stress) and the physical properties of their constituents (particle size and applesauce serum viscosity). Godfrey and others (1995) studied the effects of heating by blanching Idared and Rome apples in hot water in a

covered steam jacketed kettle at 35-83°C for 20-60 min before pulping. Applesauce was thicker at low temperature blanching (59-71°C) with the lowest USDA consistency sauce value, while little changes were observed for consistency index and applesauce serum viscosity. Yet, no effects of blanching times were found. Schijvens and others (1998) showed that heating Golden Delicious apple slices in the screw cooker with steam injection for longer time resulted in decreasing applesauce mean particle size and increasing applesauce serum viscosity, indicating higher pectin content. Additionally, at pulping step, varying screen opening size could affect applesauce consistency. Increases in screen size openings resulted in larger mean particle size (Nogueira and others 1985; Rao and others 1986; Schijvens and others 1998), and higher apparent viscosity and consistency index (Rao and others 1986; Schijvens and others 1998). Nogueira and others (1985) studied the effect of finisher rotational speed on mean particle size of R.I. Greening and Rome applesauce. Rotational speeds influenced on mean particle size depended on apple firmness. Increasing the rotational speed resulted in larger mean particle size for firm apples (firmness: 67-89 N) when changing from 500 to 900 rpm (191 to 620 N), and for soft apples (firmness: 44-67 N) when changing from 500 to 700 rpm (191 to 375 N). The reverse trend was observed for soft apples when changing at higher speed (700-900 rpm, 375-620 N). However, using R.I. Greening and Rome apples at the same firmness range (44-89 N), Rao and others (1986) found only a slight effect of speed (500-900 rpm) on flow behavior index and no effect on consistency index.

Factors Affecting the Consistency and Quality of Applesauce

Apple variety and ripening stage

Apple variety and ripening stage are the major factors for the changes of applesauce consistency since varieties of apples are used differently for processing based on their storage quality, flavor and texture. Moreover, apple physical and chemical properties (pH, total soluble solids, firmness, and pectic substances) differ by variety and change over ripening time, which can affect the flow properties of the applesauce (Rao and others 1986; Brummell 2006). Mohr (1973) and McLellan and Massey (1984) stated that different apple varieties were found to behave differently in actual particle size distribution in response to storage and ripening stage, leading to a difference in sauce consistency.

The primary changes that occur as apples mature on the tree are changes in total soluble solids (°Brix), titratable acidity, ascorbic acid, pH, flavor volatiles, starch, pectin, hemicellulose and cellulose, and related firmness values (Wiley and Binkley 1989). After harvest, ripening occurs during cold or controlled atmosphere storage, which can slow down the deteriorative processes (Bourne 1976). Bourne (1979) mentioned that during storage, apples softened moderately during ripening and showed great variability in their softening patterns depending on season, variety, climate, ripeness at harvest, and storage condition. As apples ripen, apple firmness decreases because of cells separating from each other through the middle lamella, including individual cell wall burst (Smock and Neubert 1950) coupled with the changes in the fruit chemical composition. Apple tissue texture is thought to respond

in a different manner than that of other plant tissues (Jarvis 1984) that usually degrade due to a polymerization reaction (Shewfelt 1965; Reeve 1970; Pressey and others 2008). In addition to degradation of pectin fractions in apple tissue, Knee (1978) found new soluble pectins with high degree of esterification and low levels of neutral sugar residues, leading to reduced cell cohesion, increased solubility and susceptibility to heat degradation. However, Godfrey (1993) stated that newly synthesized pectins, as reported previously, may be branched chains from insoluble pectins. Wiley and Stembridge (1961) studied the changes in texture of apple during storage and processing, as related to alcohol-insoluble solids (AIS) content. They found that starches and pectinic acid, which are the main components of AIS, decreased with maturity and storage time. Billy and others (2008) reported an increase of pectin degree of methoxylation in water-soluble pectin but decrease in alcohol-insoluble solids (AIS) extracted from Fuji and Golden Delicious during storage. From the published literature it is clear that changes in apple pectin structure and degradation due to growing conditions, variety, ripening and processing are not precisely known.

Pectic substances

Pectic substances are the major component of cell walls of fruits and vegetables and are known to contribute as gelling agents, stabilizers, thickeners, and emulsifiers of product texture. Pectin modification is a way of tailoring pectin for specific functional properties. This can be achieved by either enzymatic or chemical reactions with endogenous and/or exogenous enzymes, as well as by postharvest and/or processing dependent non-enzymatic conversion reactions. Knowledge about

pectin conversion can help to identify and track the most important enzymatic and non-enzymatic reaction mechanisms leading to pectin structural changes, and the impact of such changes in relation to physical properties of the system.

Pectin is part of a group of polysaccharides found mainly in the primary plant cell walls and the middle lamella. It is a very complex molecule that consists of several domains, the most abundant of which are homogalacturonan (HG) and rhamnogalacturonan (RG) regions. HG is the linear chain of α -(1–4)-linked d-galacturonic acid (GalA), which are methyl-esterified on the carboxyl groups (Van Buggenhout 2009). These linear structures make part of the “smooth region” of pectin, which is occasionally interrupted by “hairy regions” characterized by ramifications made up of neutral sugars. Rhamnogalacturonan type I (RG-I) is a polymer made up of alternate units of rhamnose (Rha) and GalA ramified principally with arabinan, galactan and arabinogalactan. Rhamnogalacturonan type II (RG-II) is a polymer of GalA with a complex ramification pattern (Schols and Voragen 1996; Coene 2007).

Pectin has frequently been classified into three types: water-soluble pectin (WSP) extractable with water or dilute salt solutions; chelator-soluble pectin (CSP) extractable with solutions of calcium chelating agents such as ethylenediaminetetraacetic acid (EDTA), cyclohexanediaminotetraacetic acid (CDTA), or hexametaphosphate; and protopectins that are brought into solution with alkali solutions or hot dilute acids. The pectic substances contribute both to the adhesion between the cells and to the mechanical strength of the cell wall (Jarvis 1984). The major contribution to intercellular adhesion comes from the CSP and the protopectin. The proportions of these pectin types vary considerably between different

tissues. In carrots and snap-bean pods, most of the pectin is of the chelator-soluble type (Sajjaanantakul and others 1989). In ripe and even senescent apples, most is of protopectin type (Massey and others 1964; O'Beirne and others 1982). In apples, Travakoli and Wiley (1968) found that total pectic substances accounted for 30%, and hemicellulose, hexosans and pentosans, accounted for 40% of the apple cell wall on a dry weight basis. Only galacturonic acid content varied significantly in content between apple varieties. They also found that galacturonic acid content in apples decreased between the first and fourth month after harvest, and was higher in apple varieties with greater firmness values. In general, softening during ripening (Massey and others 1964) or heating (Van Buren and others 1960) is accompanied by a loss of protopectin and an increase in water-soluble pectin.

An important factor characterizing pectin chains is the degree of methoxylation (DM) of the uronic carboxyl groups with methyl alcohol. In general, tissue pectin DM ranges from 60-90%, depending on types of fruit, variety and maturity (De Vries 1986). The pectin DM has a bearing on the firmness and cohesion of plant tissues. Based on the pectin DM, pectins are classified into low-methoxyl (<50%, LM) or high-methoxyl (>50%, HM) pectins. The formation of gels with HM pectins requires low pH (3.0-3.2) and water activity (sugar content ~65%) to form a strong network by hydrogen bonding and hydrophobic interactions (Oakenfull and Scott 1984). In contrast, the gelation in LM pectins is less pH dependent and is based on formation of calcium bridges between two carboxyl groups forming the "egg box" model (Axelos and Thibault 1991). The water insoluble pectin usually has a low percentage of methoxylation, and may be insoluble in water due to calcium bridges. In many tissues

such as in apples (O’Beirne and others 1982) and tomatoes (Burns and Pressey 1987), there are normal decreases in pectin DM that are not accompanied by firming during ripening. This could be explained by the simultaneous pectin solubilization and degradation reactions as part of the ripening and senescence processes (Sajjaanantakul and others 1989).

Pectin Methyl Esterase Activity

Pectin methylesterase (PME, EC 3.1.1.11) is an enzyme found in the cell walls of plant leaves, stems, roots, and fruit tissue (Kertesz 1951). Pectin methyl esterase hydrolyzes methyl esters from C6 of galacturonic acid (GalA) in HG and releases methanol. Doesburge (1966) reported that the groups of pectic substances mostly responsive to PME are the pectinic acids having a high degree of methoxylation. PME catalyzes the hydrolysis of methyl ester groups from the methoxylated carboxyl groups on the pectin chains, converting them into free carboxyl groups, increasing the charge density along the pectin chains, which enhances the affinity for calcium ions present in the tissues to form a fortifying network (Van Buren 1979; Burn and Pressey 1987) by forming calcium bridges (Banjongsinsiri and others 2004, Lara and others; 2004; Willats and others 2006). In addition, the free carboxyl group will increase the possibility of pectin binding to other compounds in the cell wall to increase the firmness of the tissue (Van Buren 1979). Lowering the pectin DM makes it less sensitivity to depolymerization through β -elimination, a reaction which occurs during heating of non-acid plant tissues (Sila and others 2006b) but increase its sensitivity to acid hydrolysis (Fraeye and others 2007). The action of PME is therefore a major

factor in the textural changes that occur during the fruit ripening process, which is important to determine the final texture of processed fruits and vegetables.

The demethoxylation of pectin can be accomplished by the application of exogenous PME by infiltration, or by pressure- or vacuum-assisted infusion, depending on the type of fruit (Van Buggenhout and others 2009). Many studies have been conducted and found that PME in the presence of calcium could retain or improve firmness in fruit and vegetable products such as strawberry halves (Banjongsinsiri and others 2004), pasteurized apple cubes, strawberry halves, and raspberries (Degraeve and others 2003), canned half peaches (Javeri and others 1991), strawberries (Suutarinen and others 2000), apple cubes (Guillemin and others 2006) and canned tomatoes (Grassin 2002). Fraeye and others (2009) also found a decrease of pectin DM and a substantial shift from water-soluble pectin to chelator soluble pectin due to the crosslink between pectin and Ca^{2+} when strawberries were infused with PME and Ca^{2+} at 22 °C prior to thermal processing.

Thermal stimulation can be used to activate endogenous PME. Several studies have shown that blanching at optimal conditions can influence the texture of the product as heating can enhance enzymatic reactions resulting in chemical changes in pectin structure such as pectin degree of methoxylation. It has been long known that preheating vegetables to above 50 °C can activate PME, resulting in firmer final products that were subsequently processed at high-temperature (Stolle-Smits and others 2000; Ni and others 2005). In the case of apples, thermal pretreatments at low temperatures are beneficial for the activation of PME, which seem to be variety dependent. The optimum PME temperature of 60 °C was reported for Bramley (King

2007), 55 °C for Golden Delicious and York Imperial (Lee and Wiley 1970), 59 °C for Idared and 59-71 °C for Rome apples (Godfrey and others 1995). In addition, the amount of time the product is held at these temperatures affects the degree of firming, with longer times at lower temperatures leading to a greater degree of firmness (Van Buren and others 1960).

Applesauce particle size distribution

It is reported that rheological properties of fruit puree are influenced by pulp content, particle size and the interactions between particles (Mizrahi and others 1970; Marsh and others 1980; Beresovsky and others 1995; Yoo and Rao 1994,1995). Changes in the rheological properties of fruit purees can be accomplished by changing the pulp concentration (Tanglertpaibul and Rao 1987; Yoo and Rao 1994,1995; Schijvens 1998; Ouden and Van Vliet 2002) and the particle size distribution (PSD) (Lopez and others 1996; Pickardt and others 2004). Homogenization in apricot puree (Duran and Costell 1985) and using different sizes of finisher screen openings in tomato puree (Tangerlertpaibul and Rao 1987) could lead to particle size changes. The effect of particle size on rheology can be explained by recognizing that as the particle size decreases, the number of particles in a given volume increases, resulting in a decrease in the mean distance between the particles; thus increasing the potential for particle-particle interaction (Agarwala 1992). However, studies that relate particle size to rheological properties of applesauce are often contradictory. Missaire and others (1990) observed an increase in yield stress with an increase in the mean particle size while Qiu and Rao (1988) found an increase in yield stress with a decrease of mean

particle size. Changing applesauce processing parameters - screen opening size, finisher rotational speed, thermal treatment – resulted in modifying applesauce mean particle size and pulp content (Qiu and Rao 1988; Schijvens and others 1998). Larger screen sizes caused larger mean particle size and higher pulp content while longer heating resulted in smaller mean particle size and higher pulp content.

Thermal treatment

Thermal processing such as cooking, pasteurization, and sterilization of fruits and vegetables is a convenient and efficient way to produce goods that can be stored for extended time. Heat processing of fruits causes several structural changes resulting in product softening (Aguilera and Stanley 1990). The softening occurs as a result of solubilization and depolymerization of pectic polymers that are involved in cell-cell adhesion (Van Buren 1979; Greve and others 1994; Cano 1996; Waldron and others 1997, 2003). Chemical pectin depolymerization during heat treatments can be explained by β -elimination and/or acid hydrolysis depending on the degree of methoxylation (DM) and the pH of the system. Pectin with a high DM is more subject to β -elimination than pectin with a low DM (Sajjaanantakul and others 1989, 1993; Krall and McFeeters 1998; Fraeye and others 2007). β -elimination and acid hydrolysis of pectin have been measured, respectively, down to pH value of 3.8 and up to pH value of 6 (Smidsrod and others 1966; Krall and McFeeters 1998).

Thermal processing is also related to the changes in pectin solubility in plant-based foods. Heat could cause a substantial increase in pectin solubility (Plat and others 1988; Ben-Shalom and others 1992), and specifically an increase in water-

soluble pectin content (Nyman and others 1993; Greve and others 1994; Sila and others 2005, 2006a, 2006b). The pectin solubilization can be explained by two processes; (1) the polymer is depolymerized via the β -elimination reaction making the molecules small enough so that they no longer bind to the cell-wall gel framework (Rees 1972) followed by (2) partial solubilization, which is probably due to the fragmentation of the hairy region and cleavage of the backbone (Plat and others 1988). Consequently, the aggregation of pectin molecules is reduced and the association with other cell wall polymers is broken, resulting in the release of pectin to the cell matrix (Massiot and others 1992; Kunzek and others 1999).

Little has been reported on parameters affecting the consistency of applesauce. Therefore, additional studies are needed to further assess the effect of apple variety, maturity and processing conditions on the rheological properties and quality of applesauce.

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CHAPTER 3

HEAT TREATMENT AND TURBO EXTRACTOR ROTATIONAL SPEED EFFECTS ON RHEOLOGICAL AND PHYSICO-CHEMICAL PROPERTIES OF VARIETAL APPLESAUCE

Abstract

We studied the effect of apple variety, ripening and processing parameters on applesauce rheology. Four varieties at 3 maturity stages were processed into applesauce. Apples were diced, heated to 85°C for hot break sauce (no heating for cold break sauce), fed to a turbo extractor (400-1800 rpm) and hot-packed. Samples were analyzed for rheological, physical and chemical properties. Results were analyzed by ANOVA and Tukey's test ($p \leq 0.05$). Variety, ripening, extractor speed, and heat treatment significantly affected sauce properties. Increasing speed produced thicker sauce. Ripening improved consistency for Crispin and Cortland cold break sauces. Hot break sauce had consistent sauce quality over time with less syneresis, higher pectin content, smaller mean particle diameter, and wider particle size distribution, compared to cold break sauce. Thus, heating apples before pulping could be used to overcome variations in consistency associated with variety and ripening. Consistency and free-liquid flow could be predicted as a function of particle size, pectin content and pectin degree of methoxylation ($R^2 = 0.80$ and 0.93).

Introduction

Apples are grown throughout the United States and represent an important economic crop to states with large production such as Washington and New York. In 2010, the production of apples in the United States was 4.18 million tons, valued at 2.22 billion dollars of utilized production. A third (34.4%) of apples are processed into juices, concentrates, purees or applesauce (USDA 2012). Applesauce consumption has increased due to consumer demands for healthier, ready-to-use foods, as it constitutes a convenient alternative to candy and chocolate bars for children (Colin-Henrion and others 2009).

An important quality attribute of applesauce is its consistency, which is dependent on chemical components and physical properties such as size and distribution of apple tissue particles. These components can be affected by many factors, including variety and ripening of the fruit and any operations involved in post-harvest handling, storage and processing (Mohr 1989). Previous studies on applesauce have focused on how the rheological properties relate to the processing parameters applied. Godfrey and others (1995) studied the effect of heating by blanching Idared and Rome apples in hot water at 35-83°C for 20-60 min before pulping to sauce. They found a decrease in USDA consistency values (thicker sauce, using a consistometer chart No. 1) at low temperatures (59-71°C) while values increased (thinner sauce) at higher temperatures, and no significant changes were reported for consistency index and applesauce serum capillary viscosity. The effect of blanching time was not significant, and the thickening effect was believed to be caused by pectin methylesterase (PME) activity. In addition, Schijvens and others (1998) reported that,

when heating Golden Delicious apple slices in a screw cooker with steam injection, longer cooking times resulted in increasing applesauce apparent viscosity, applesauce serum capillary viscosity and water insoluble solids, but decreasing applesauce mean particle size. At the pulping step, increasing finisher screen opening size resulted in larger mean particle size (Nogueira and others 1985; Rao and others 1986; Schijvens and others 1998), leading to higher apparent viscosity and consistency index (Rao and others 1986; Schijvens and others 1998). However, increasing finisher speed (500-900 rpm) caused an increase in mean particle size (Nogueira and others 1985) but no effect on consistency index (Rao and others 1986) with the same apple varieties and similar firmness ranges. Therefore, the processing-induced changes in applesauce rheology were likely resulting from changes in chemical and physical properties and seem to be very specific to the processing parameters involved. Colin-Henrion and others (2009) have studied processing effects on dietary fibers and cell wall polysaccharides in applesauce, yet without information on applesauce consistency and particle size distribution.

In addition to processing, the maturity stage of the fruit and the apple variety may affect applesauce consistency. Variations in raw material composition affect industrial processing of fruits, as nowadays apples are processed all year long due to availability from cold storage (0-2°C, 90-95%RH) and controlled atmosphere (CA) storage (-1-4°C, 1-3% O₂, 4% CO₂) (Hardenburg and others 1986; USDA 2012). Impact of storage on apple composition is well documented, and the main changes include loss of neutral sugars, solubilisation and depolymerization of cell wall polysaccharides due to the combined action of several cell-wall-degrading enzymes on

pectic and cellulosic fractions (Brummell 2006; Goulao and Oliveira 2008). The effect of these changes on applesauce processing is yet to be determined. Additionally, commercial applesauce processing methods have changed from hot break to cold break process with the utilization of turbo extractors, which can pulp the whole fruit to sauce or puree at room temperature without any thermal pretreatment. Understanding the influence of both raw materials and processing conditions on sauce properties will help to develop food products with improved quality. Our goals were to study the impact of apple variety, ripeness, and key processing parameters (heat treatment and extractor rotational speed) on rheological properties of applesauce, following current industrial procedures, and to model the relationship between physical, chemical and rheological properties of applesauce.

Material and Methods

Materials

Four apple varieties (*Malus domestica*) from the 2010 harvest, Crispin, Cortland, Jonagold, and Idared were used for the study. Apples were sourced from upstate New York farms and had been kept in controlled atmosphere (CA) storage (-1-2°C, 1-3% O₂, 4% CO₂) for 6-8 months. Immediately after receiving the fruit at the New York State Agricultural Experimental Station (Geneva, NY), apples were placed in cold storage at 10°C and 95% relative humidity (RH) to accelerate fruit ripening. Apples were processed into applesauce at three different ripening times; 0 (beginning), 18 (middle) and 36 (matured) days of storage.

Prior to processing, apples were weighed and tested for firmness using a hand-held penetrometer model FT 327 (Wagner Instruments, Greenwich, CT). Apples were processed into applesauce following procedures described in Figure 3.1. Apples (~ 15 kg) were diced (1.27 cm) by a Dicer Model No.C (Urschel Laboratories Inc., Valparasio, IN). Apple dices were steam heated in a Vulcan Steam Oven (Model 3TE; Diagon Devices, Inc., Pullham, WA) to 85°C for 16 min (representing hot break sauce), and pulped by a Turbo extractor (Bertocchi CX5, Bertocchi SLR., Parma, Italy) fitted with a 3.2 mm opening screen at three extractor rotational speeds: 400 (low), 800 (medium) and 1300 (high) rpm. The cold break sauce was unheated and pulped by the Turbo extractor, at higher rotational speeds due to the firmness of uncooked dices, at 800 (low), 1300 (medium) and 1800 (high) rpm. Water was added (10% and 15% for hot and cold break sauces, respectively) to standardize the soluble solids, and a small amount of 10% ascorbic acid solution was added to prevent browning prior to pasteurization. Sauce was pasteurized ($t = 6$ min, $T=90^{\circ}\text{C}$) in an agitated steam kettle (Groen MFG. Co., Chicago, IL), and hot-filled into 16 oz. glass jars. Jars were immediately capped, turned upside down for 2 min for cap sterilization and quickly cooled in a water bath to room temperature.

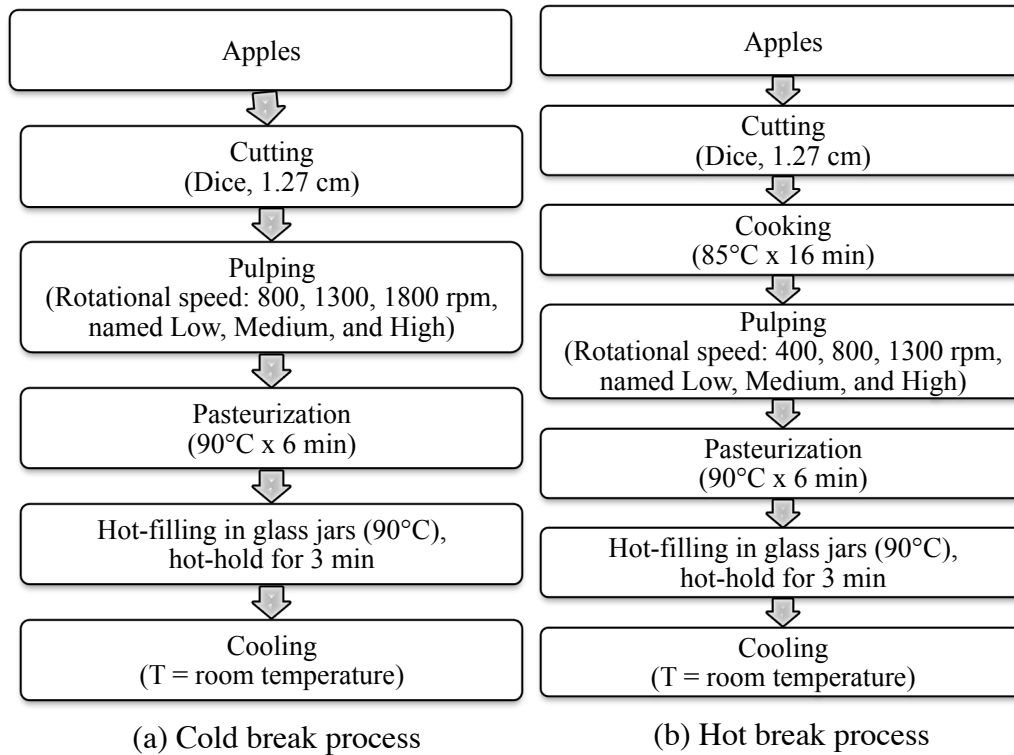


Figure 3.1 Flow chart of (a) cold and (b) hot break applesauce processing

Rheology measurements

The measurements were carried out on a Brookfield DV-III Ultra programmable rheometer (Brookfield Engineering Laboratories, INC. Middleboro, MA) with V73 plus spindle to obtain values of shear stress for each applesauce sample. The measurements were determined by applying shear rate from 0.5 to 3.0 s^{-1} and back at 25°C during a total time of 11 minutes, and taken every minute. Then the consistency index and yield stress were determined for each samples using Power law Model and Casson's Model. Based on the Grading Manual for Canned applesauce

(USDA 2005), USDA consistency values for sauce and liquid were measured at room temperature using the USDA Consistometer flow sheet no.1, by averaging sauce and liquid spread of products subjected to flow for 1 minute on a plastic chart containing concentric circular markings, 0.5 cm apart. USDA consistency free-liquid is achieved by subtracting sauce from liquid flow.

Particle size measurements

Particle size distribution (PSD) measurements were performed using laser diffraction (Mastersizer model MS-2000, Malvern Instrument Inc., Westborough, MA). Applesauce samples were added into a water-continuous dilution accessory (2000 Hydro-S) filled with deionized water. The particle size distribution was calculated from the intensity profile of the scattered light using the instrument software (Mastersizer 2000, version 5.40). The volume based (d_{43}) mean particle diameter and the width of distribution (span) were obtained for every sample:

$$d_{43} = \sum_i n_i d_i^4 / \sum_i n_i d_i^3 \text{ where } n_i \text{ is the number of particles of diameter } d_i$$

Equation 3.1

$$\text{Distribution span} = (d_{90\text{th percentile}} - d_{10\text{th percentile}}) / d_{50\text{th percentile}}$$

Equation 3.2

pH, total soluble solids, moisture and titratable acidity

Applesauces were measured for pH using a pH meter (Accumet Basic AB15 pH meter; Fisher Scientific Waltham, MA). Total soluble solids content (TSS) of applesauces was measured with a Leica Auto ABBE refractometer (Leica Inc.,

Buffalo, NY) and reported as °Brix. Applesauce moisture was obtained according to AOAC (2000). Applesauce serum was collected after centrifuged at 41,840 x g (17,000 rpm) for 30 min (Sorvell SA-600 Centrifuge; ThermoScientific, Waltham, MA). Titratable acidity of applesauce serums was measured by a G20 compact titrator (Mettler-Toledo International Inc., Columbus, OH) using 0.1 N NaOH as a titrant to an endpoint at pH 8.10. TA was calculated and reported as an equivalent weight of g malic acid per 100 g applesauce serum.

Total soluble pectin content (TSP) and its degree of methoxylation

For alcohol insoluble residue (AIR) assessment, applesauce was homogenized with 3 volumes of 95 % ethanol. The precipitate was filtered through Whatman filter paper 55 mm (Whatman, Piscataway, NJ), dried in oven at 70 °C until stable, weighed and recorded as % AIR. Soluble pectin content (as galacturonic acid equivalent) was determined according to Fraeye and others (2009) and pectin degree of methoxylation was determined by using alcohol oxidase and Purpald as described by Anthon and Barrett (2004) in water-soluble and chelator-soluble pectin fractions of applesauce, which were proportionally combined as fractions of total soluble pectin, and overall degree of methylation of sauces.

Statistical Analysis

Two apple batches were processed for each processing treatment, and two jars of applesauce were collected for each batch. Statistical analysis was performed using JMP 10.0 (SAS Institute Inc., Cary, NC), and throughout the paper we control the

probability of Type I error at the 5% level. Before running the ANOVA and regression models, we tested for potential multicollinearity and performed Principal Component Analysis (PCA) for rheological properties and chose the number of components so that 90% of variability is explained by the top K components. Since the variables showed strong correlations, this step was essential to increasing the power of our analysis. To test differences between mean effects of apple variety, ripening days, extractor speed and cooking treatment, we used Tukey's Honestly Significant Difference (HSD) at 95 % confident interval. Then PCA was used to calculate multivariate product spaces to examine all variable effects and hierarchical ascendant classification (HAC) was performed. Finally, we selected the final model to predict rheological properties based on the maximal Bayesian Information Criterion (BIC) in a stepwise regression procedure.

Results and Discussion

Apple and applesauce analysis

Firmness could be used as an indicator for apple ripening stage as the softening in apple tissue resulted from modifications of chemical and physical components during ripening (Johnson and others 2002). In this study, during accelerated ripening at 10°C for up to 36 days, apple firmness significantly decreased from 50-60 to 35-40 N for Cortland and from 58-62 to 45-50 N for Crispin, showing the change from fresh to ripened stage. However, there was no significant change for Idared (40-50 N) and Jonagold (38-45 N). Mohr (1989) reported that Idared apples could maintain good

firm fruit condition for a very long time (8 months or longer at 0°C air storage) and notably resist cell wall separation. Thus, Idared apples firmness did not change within the range of this study but it should be noted that a decrease in firmness might be observed if ripening times were longer. However, Jonagold firmness after CA storage was lower than values from fresh apples at the same harvest year reported by another study by our group (60-65 N). Therefore, Jonagold apples may have ripened during CA storage and at day 36, apples were believed to fall in the overripe stage. Applesauce had 9.5-11.5 °Brix, pH 3.4-3.8, titratable acidity 0.3-0.5% and moisture content 86-88% depending on apple variety and ripening stages. Firmness and other fruit ripening indicators and trends were in agreement with previous literature (La Belle 1981; Massey Jr. 1989). During ripening, most applesauce, except for Jonagold, had higher soluble solids and pH due to higher sugar levels and loss of acid, respectively. The lower soluble solids for Jonagold sauce supported the hypothesis that Jonagold apples went to senescent stage. Additionally, no effect of heat treatment and extractor rotational speed were found on soluble solids, pH, and moisture content.

Rheological properties

Rheological properties of applesauce were well explained with more than 95% confident of fit by power law model with the parameter, consistency index, and Casson's model with the parameter, yield stress. Those two models have been previously used for the description of applesauce and apple puree (Rao and others 1986; Qiu and Rao 1988; Godfrey and others 1995; Cantu-Lozano and others 2000). All applesauce in this study had consistency index and yield stress ranging 30-270

Pa.s, and 30-160 Pa, depending on variety, ripening days and cooking treatment and those values were within the range reported by previous studies (Rao and others 1986; Qiu and Rao 1988; Godfrey and others 1995).

Applesauce rheological properties were described by the first two components of the Principal Component Analysis (PCA), which accounted for 68% and 25% of the total sum of squares and kept for analysis (Table 3.1). The first two components were named consistency component, which is positively related to high viscosity, and free-liquid flow, which is linearly related to USDA consistency free-liquid. The desired applesauce quality should have a high consistency component and low free-liquid flow. Apple variety, ripening days, extractor rotational speed, heat treatment and their interactions significantly affected consistency component and free-liquid flow ($p < 0.0001$).

Table 3.1 Eigenvectors of principle component analysis of applesauce rheological properties by first two components

Parameters	Consistency component	Free liquid flow
Power law consistency index	0.59	0.03
Casson's yield stress	0.59	0.05
$1/(\text{USDA consistency sauce})^2$	0.55	0.07
USDA consistency free-liquid flow	-0.08	1.00

- Effect of turbo extractor rotational speed

The effect of extractor rotational speed on consistency component and free-liquid flow was similar for all varieties and ripening days. Therefore, Figure 3.2 shows

the averaged values of consistency component and free-liquid flow from all varieties and ripening days by extractor rotational speed for cold and hot break sauce. For cold break sauce, consistency component significantly increased and free liquid flow decreased when extractor speed increased from low to medium with 1-1.5 times higher consistency index, 1-4 times higher yield stress, 10-50% lower USDA consistency sauce and 10-20% lower USDA consistency free-liquid. No significant change was observed when changing from medium to high speed. For hot break sauce, consistency component increased significantly from low to high speed with 1-2 times higher consistency index and yield stress, and 10-20% lower USDA consistency sauce. However there was no change for free-liquid flow (Figure 3.2).

The extractor rotational speed is correlated to centrifugal force, which is the driving force to push apples against the screen ($F \propto \text{extractor rotational speed}^2$) (McLellan 2005). The high turning speed (high force) lets the product explode and quickly release the liquid and pulpy parts through the screen. At low speed for cold break sauce, force was insufficient to destroy apple cell wall and push apples through the screen; as a result, less pulpy parts were added to the extractor output, causing low consistency component and high free liquid flow. Speed had less impact once the force reached the maximum threshold, explaining why less changes were observed between medium and high speed. Hot break sauce was generally less effect by speed as it required less force to push products through the screen since apples were softened during heat treatment. However, inappropriate speed for hot break sauce could result in undesirable products as found in this study. At low speed for hot break sauce, product turned out to be pulpy apple juice when processed from high firmness apples

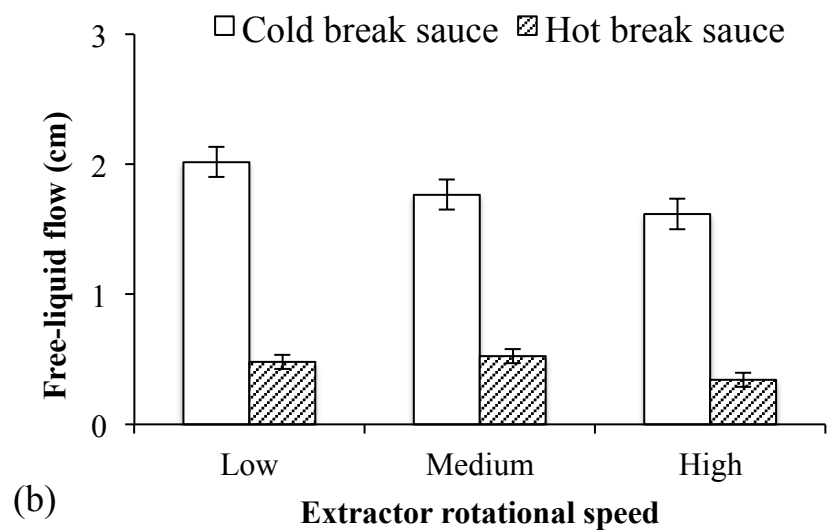
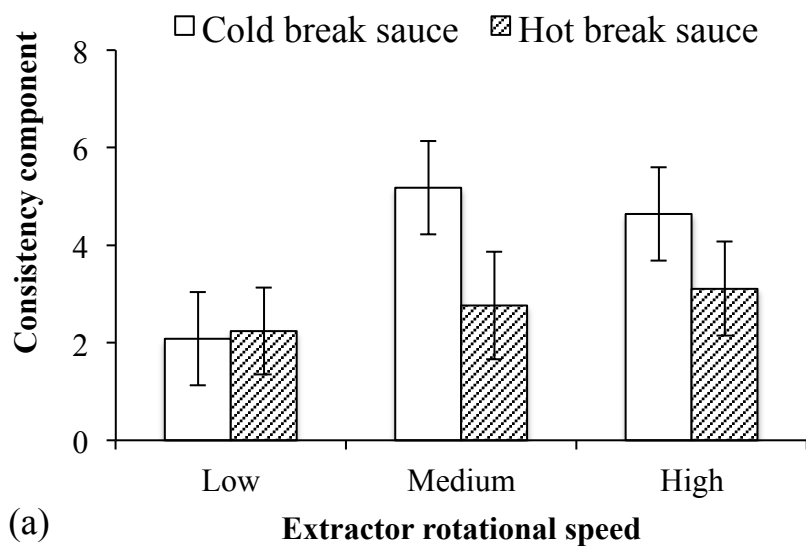


Figure 3.2 Effect of extractor rotational speed on (a) consistency component (dimensionless) and (b) free-liquid flow of cold and hot break applesauce averaged from four varieties and three ripening days

(Crispin, Cortland) since apple dices were not softened enough from heating and less force was applied at extracting step. Therefore, producers should select optimum speed based on high firmness apples and ensure that apple dices have been softened enough during heating. Due to the highest consistency component and lowest free-liquid flow, applesauce processed at high speed for both cold break (1800 rpm) and hot break sauces (1300 rpm) were chosen to conduct further chemical analysis.

- Effect of heat treatment and ripening

The effect of heat treatment on consistency component and free-liquid flow depended on ripening days and apple variety. For Cortland and Crispin cold break sauce, increasing ripening days from 0 to 36 significantly increased consistency component, and decreased free-liquid flow, showing better sauce quality (Figures 3.3 and 3.4). Cold break applesauce processed at day 36 had 1-2 times higher consistency index and yield stress, 8-16% lower USDA consistency sauce and 50-70% lower USDA consistency free-liquid than from day 0 for Cortland, and 3-4 times higher consistency index and yield stress, 10-16% lower USDA consistency sauce and 80-100% lower USDA consistency free-liquid for Crispin. However, there was no significant difference on consistency component and free-liquid flow for both Idared and Jonagold cold break sauces, indicating there was no effect from ripening on sauce processed from these two varieties. However, free-liquid flow for Jonagold cold break sauce increased with ripening days, showing that sauce processed from overripe apples had worse quality than ripened apples. It should be noted that the change in consistency component and free-liquid flow produced the same results as the change in apple firmness. Thus, apple firmness could be used as a quick indicator to track the

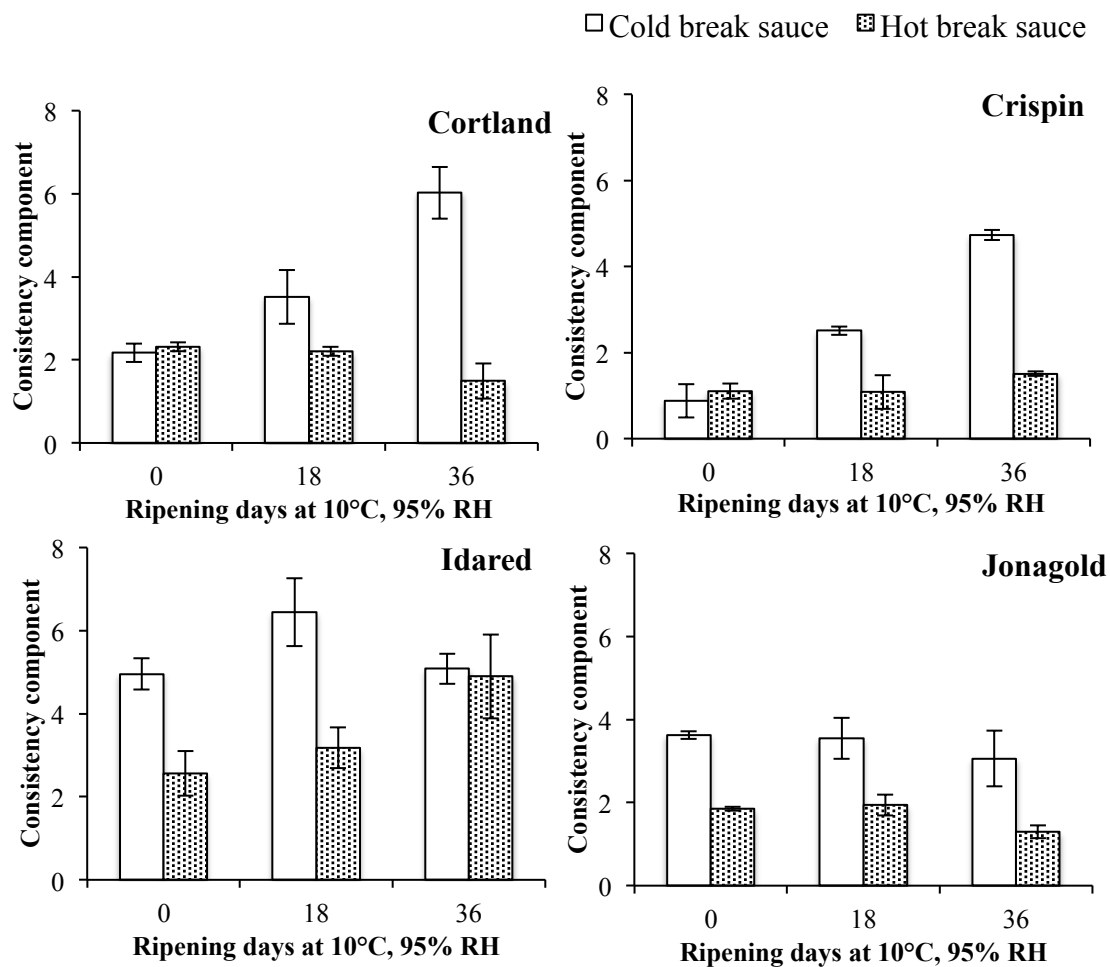


Figure 3.3 Consistency component (dimensionless) of varietal applesauce made from cold break process (rotational speed 1800 rpm) and hot break process (rotational speed 1300 rpm)

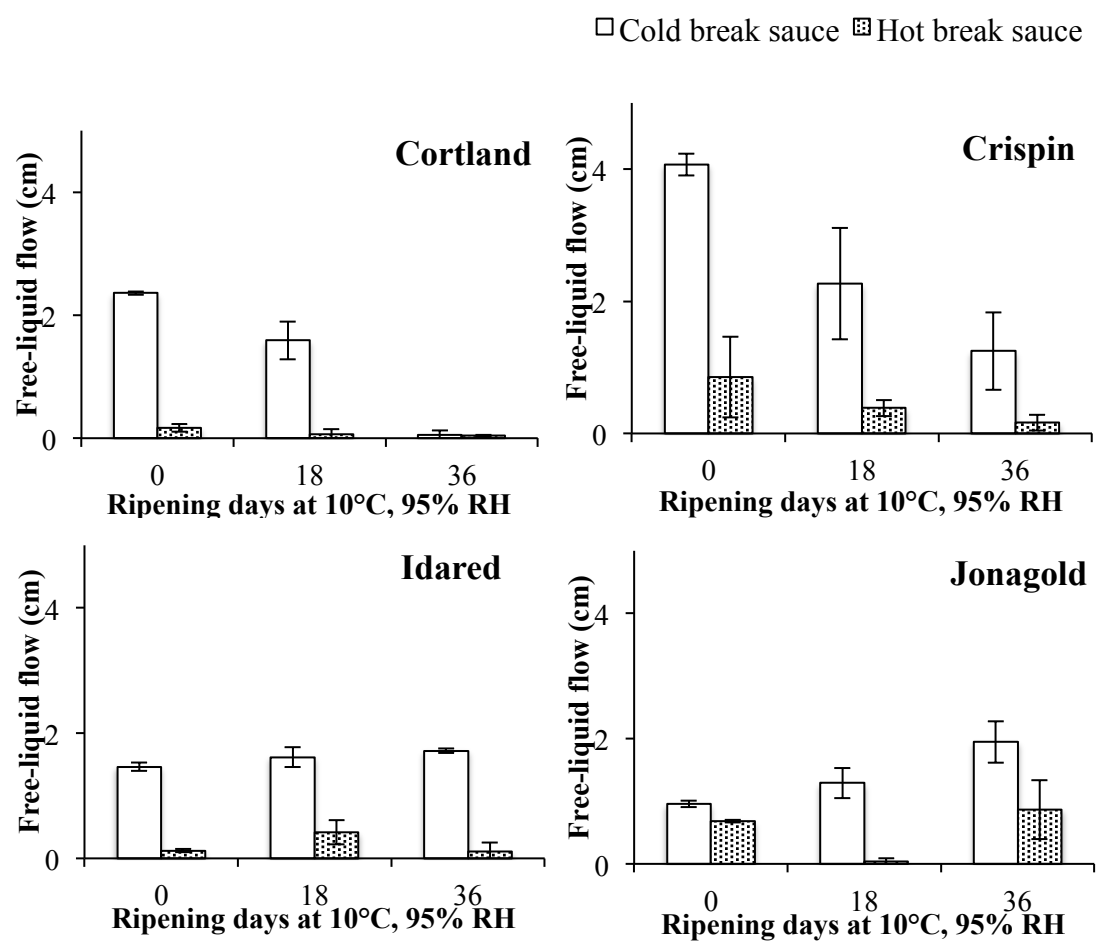


Figure 3.4 Free-liquid flow of varietal applesauce made from cold break process (rotational speed 1800 rpm) and hot break process (rotational speed 1300 rpm)

change in applesauce rheology. Apple varieties responded to accelerated ripening differently and therefore applesauce samples produced over time showed consistency differences.

Compared to cold break sauce processed at the same ripening day, hot break sauce had lower consistency component when processed from ripened apples (30-70% reduction of consistency index and yield stress and 15-30% increase in USDA consistency sauce), while no significant difference was observed when processed from fresh apples (Figure 3.3). Hot break sauce had 60-100% significantly lower free-liquid flow for all applesauce, regardless of variety and ripeness (Figure 3.4). We can see that the quality of applesauce made from Idared and Jonagold, which could not be improved by ripening, could be modified by the heating treatment. Waldron and others (1997) reported that if the forces holding a cell together were stronger than the cell walls, then failure occurred in the cell walls; if the forces holding the cells together were weaker than the cell walls, then the cells will separate. Therefore, in firm, unheated apples, cell adhesion was strong and tissue fracture involved rupture across the cell contents; hence cell contents were released and became juiciness. While processes such as heating or fruit ripening resulted in a considerable softening of apple tissue and decreased cell adhesion, the rupture of tissue occurred by cell separation with no destruction of cell wall materials and no cell content released, causing less free-liquid separation. The same results were found in carrots and broccoli where single cells and smaller clusters were observed for the hot break samples, showing the cell separation, compared to cold break samples (Lopez-Sanchez and others 2011). Additionally, hot break sauces were less affected by apple ripening stage as both

rheological properties had similar values for all ripening days within the same variety, thus heat treatment could be used to overcome the variation associated with apple ripeness. The change in applesauce rheology due to both ripening and heating effects were generally accompanied by changes in particle size distributions and chemical composition.

Particle size distributions (PSDs)

The extractor rotational speed, apple variety, ripening days, heat treatment and their interactions had significant effects on volume weighted mean diameter (d_{43}) and distribution span. For all varieties and ripening days, increasing speed from low to medium for cold break sauce caused an increase of 6-12% d_{43} and a decrease of 6-13% distribution span due to the addition of more apple pulp to the final product. There was no effect of extractor speed on d_{43} and distribution span for hot break sauce.

PSDs of cold break sauces exhibited sharp and narrow unimodal distributions (1.3-2.0 for distribution span) and higher d_{43} (680-950 μm) while the hot break sauce had a bimodal distribution (2.0-2.8 for distribution span) and lower d_{43} (400-650 μm) (Figure 3.5). For cold break sauce, particle size characteristics depended on apple variety and ripening stages. Increasing ripening days from 0 to 36 resulted in 10-40% decrease of d_{43} and 5-25% increase in distribution span for ‘Crispin’ and ‘Cortland’. However, the opposite result was found for Jonagold (10-25% higher d_{43} and 5-25% lower distribution span) and there was no difference for Idared. Compared to cold break sauce processed from the same variety and ripening days, hot break sauces had significantly 30-80% lower d_{43} and 20-45% higher distribution span. In addition, their

particle size distributions were not significantly different over ripening within the same variety, except for Jonagold at day 36. The difference observed for Jonagold applesauce might be the result from using over ripe apples. Similar results were found in previous studies by Vetter and others (2001) as they reported that heated apple cell wall material had smaller mean particle diameter than unheated apple cell wall material. A higher number of smaller size particles could enhance particle surface-surface contacts and decrease the mean distance between the particles, increasing the potential of particle-particle interaction (Agarwala and others 1992; Yoo and Rao 1994; Afoakwa and others 2008); thus this could result in higher consistency component and less free-liquid flow, as found in cold break sauce made from ripened apples. The lower consistency component for hot break sauce compared to cold break sauce could be explained by the smaller d_{43} and the wider particle distribution pattern. The maximum packing fraction increased due to the change to a bimodal distribution, as the small particles were the correct size to fit between the larger particles. An increase in the maximum packing fraction of a system means that there is more free volume available for the particles to move around in, therefore fewer particle-particle interactions and less resistance to flow, hence a lower consistency (Greenwood and others 1997). However, even though a shift to a smaller mean particle diameter was observed with ripening, the cold break sauce still had a higher consistency component. A possible explanation is that the smaller particles found in cold break sauce were still too large to fit between the larger particles, and the distribution pattern remained unimodal; thus, the maximum packing fraction remained the same. Additionally, the change in total soluble pectin may affect the rheological properties, which will be

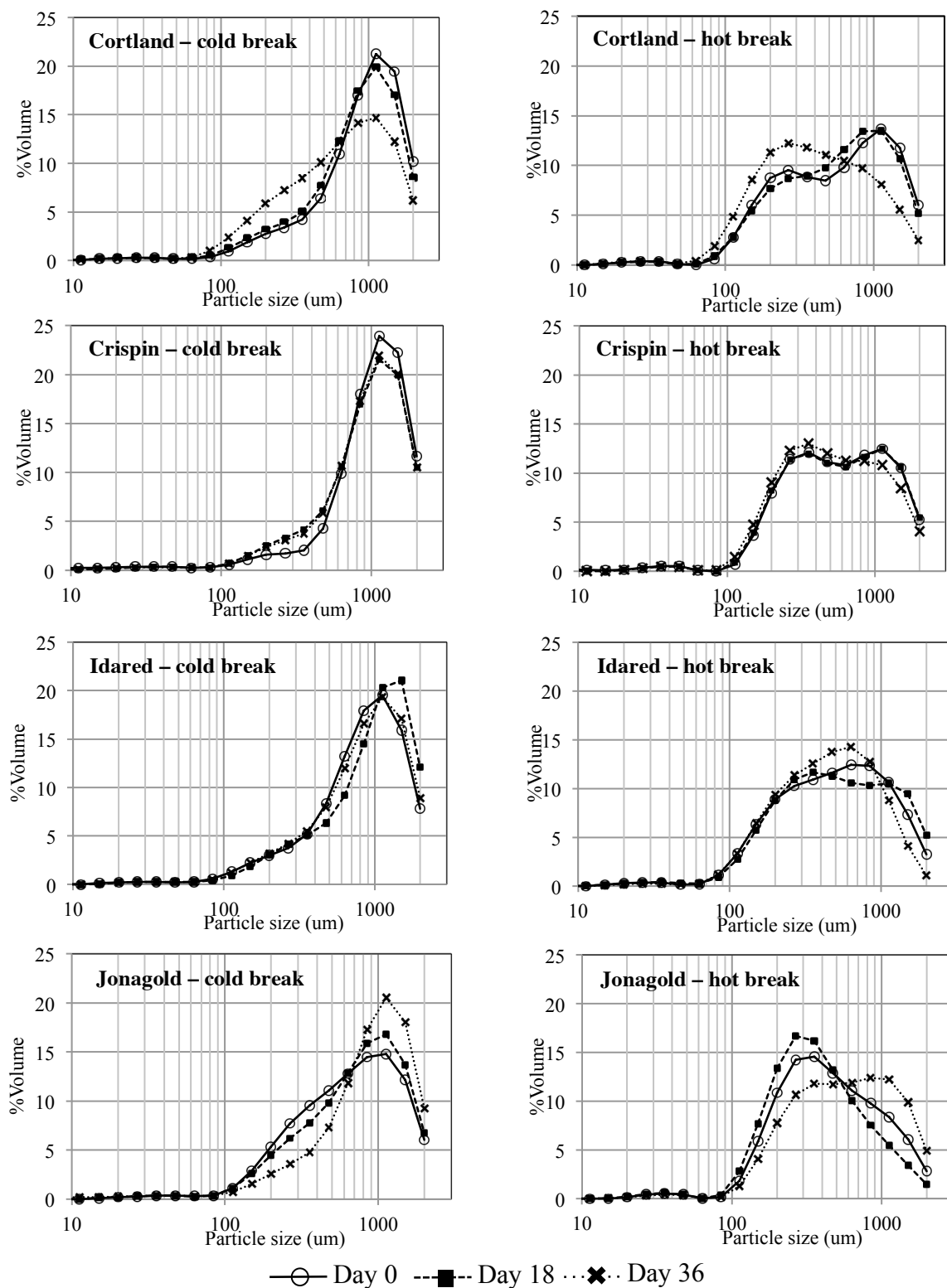


Figure 3.5 Particle size distribution of varietal applesauce made from cold break process (rotational speed 1800 rpm) and hot break process (rotational speed 1300 rpm)

described in the next section.

Total soluble pectin (TSP) and pectin degree of methoxylation (DM)

Pectin changes that occur during ripening and heat treatment are relevant to applesauce consistency and free-liquid flow. The TSP and pectin DM values measured in varietal applesauce are given in Table 3.2. TSP varied significantly with Idared applesauce containing the highest amount, followed by Cortland, Crispin, and Jonagold. Range of results (0.14 – 0.33%) and trends observed were similar to those reported in the literature (0.17 – 0.55%) for apples, over apple storage time, and for varietal applesauce (De Vries 1981; Scalzo and others 2005; Vanoli and others 2009; Le Bourvellec and others 2011). The general trend was that during ripening, TSP increased significantly for Cortland and Crispin cold break sauce, while no significant change was observed for Idared and Jonagold. Nevertheless, a decrease in TSP was found for Jonagold cold break sauce, indicating that apples were over ripe as reported by a previous study (Lozano 2006). During the ripening process, pectin undergoes extensive structural changes associated with the general decrease in firmness and loss of cell cohesion (Bartley and Knee 1982; Redgwell and others 1997). This could explain the decrease in firmness for Crispin and Cortland. The main structural changes include the rupture of pectin bonds to other cell wall polysaccharides and depolymerization of the galacturonate backbone, resulting in the conversion of protopectin to soluble pectin. Those reactions are caused by enzymes, including endo- and exo-polygalacturonase and glycosidase (Wallner and Walker 1975; Gross and Wallner 1979; Gross and Sams 1984; Billy and others 2008). Pectin solubility may

also be increased by cleavage of linkages between side chain of pectin and hemicellulose (Nogata and others 1996). According to Table 3.2, there was no significant difference on TSP from heat treatment for all four varieties and three ripening days; however, TSP values in hot break sauce were higher than cold break sauce. An increase in TSP from heating was reported by Plat and others (1998) and Ben-Shalom and others (1992). Heat could solubilize pectin polymers, similarly to ripening, through a non-enzymatic β -elimination reaction (Sakai and others 1993; Voragen and others 1995; Thakur and others 1997). The higher TSP contributes to higher consistency in applesauce as it has been reported that the losses in pectin due to heating result in lower viscosities in tomato purees and formulated tomato products (Van Buggenhout and others 2009).

The values for the pectin DM in applesauce are shown in Table 3.2. Our results (33-70%) were comparable to those reported in the literature (47-88%) for apple varieties, with progress of storage time and for varietal applesauce (De Vries and others 1984; Klein and others 1995; Johnston and others 2002; Anthon and Barret 2008; Rascón-Chu 2009; Le Bourvellec and others 2011). Ripening and cooking treatment did not have a significant effect on pectin DM for most varieties except for Crispin applesauce (pectin DM > 60%), which showed a significant decrease during ripening (15-30%) in cold break sauce. Pectin DM was strongly depended on apple variety, which could lead to different sauce properties despite the similar pectin content.

Table 3.2 Total soluble pectin (g galacturonic acid equivalent per 100 g applesauce) and pectin degree of methoxylation (%) of varietal applesauce made from cold break process (1800 rpm rotational speed) and hot break process (1300 rpm rotational speed)

Variety	Ripening days	Total soluble pectin (%)		Pectin degree of methoxylation (%)	
		Cold break sauce	Hot break sauce	Cold break sauce	Hot break sauce
Cortland	0	0.18 ^{cde}	0.20 ^{bcd}	55 ^{abcd}	60 ^{ab}
	18	0.22 ^{bcd}	0.22 ^{bcd}	56 ^{abcd}	55 ^{abcd}
	36	0.33 ^a	0.22 ^{bcd}	55 ^{abcde}	58 ^{abc}
Crispin	0	0.14 ^c	0.15 ^c	65 ^a	59 ^{ab}
	18	0.16 ^{de}	0.17 ^{cde}	56 ^{abc}	53 ^{bcdef}
	36	0.21 ^{bcd}	0.20 ^{cde}	48 ^{bcd}	46 ^{cdefgh}
Idared	0	0.26 ^{abc}	0.32 ^{cde}	40 ^{ghi}	44 ^{defghi}
	18	0.26 ^{abcd}	0.33 ^{cde}	42 ^{fghi}	39 ^{ghi}
	36	0.30 ^{ab}	0.33 ^{cde}	35 ^{hi}	40 ^{ghi}
Jonagold	0	0.21 ^{abc}	0.20 ^a	33 ^{hi}	38 ^{ghi}
	18	0.19 ^{abcd}	0.16 ^a	40 ^{ghi}	37 ^{ghi}
	36	0.14 ^{ab}	0.16 ^a	39 ^{ghi}	43 ^{efghi}

Values followed by different letters differ significantly at the 0.05 level (Tukey's HSD test compared by variety, ripening days and cooking effect)

Relationships between rheological properties, particle size characteristics, pectin contents, and pectin DM

PCA was performed to correlate the physical, chemical and rheological properties of applesauce. The first three components explained 92% of the variability in the data. The first two components are presented in Figure 3.6a. TSP and pectin DM had polar influences on PC3 (12.7% variation). Along PC1, free-liquid flow is positively correlated to d_{43} ($r = 0.83$) and negatively correlated to distribution span ($r = -0.81$). Further examination of the loading plot between PC2 and PC3 shows that free-liquid flow is also correlated to TSP ($r = -0.38$) and pectin DM ($r = 0.20$). The second

component represents the consistency component, which positively correlated with total soluble pectin ($r = 0.61$) and negatively correlated with pectin DM ($r = -0.37$). Additionally, the consistency component, when compared between PC1 and PC3, is correlated to mean particle diameter (d_{43}) ($r = 0.33$) and distribution span ($r = -0.42$). A high correlation was found between distribution span and d_{43} ($r = -0.92$), showing that when applesauces have smaller mean particle diameter, they normally have wider size distribution.

The HAC performed on the PCA data outlined three groups of applesauce products (shown by ellipses in Figure 3.6b). The first group consisted of cold break sauce processed from less ripened (Cortland and Crispin at day 0, 18) and overripe apples (Jonagold at day 18, 36). These applesauces were characterized by having low consistency component, high free-liquid flow, and low pectin content. The second group was cold break sauce processed from ripened apples (Cortland, Crispin at day 36, Jonagold at day 0, Idared at day 0, 18, 36) and hot break Idared sauce. These applesauces had the highest consistency component and pectin content, but showed a wide range of free-liquid flow. The third group was composed of hot break sauce processed from Cortland, Crispin and Jonagold for all three ripening days. They had the lowest consistency component and free-liquid flow. The results clearly indicate that hot break sauces were less affected by variety and ripening days.

Since PCA showed that consistency component and free-liquid flow had high correlation with distribution span, d_{43} , TSP and pectin DM, the change in rheological properties might be attributed to the change in these explanatory variables and their interactions. All data from both hot and cold break sauce were subjected to stepwise

regression to predict consistency component and free-liquid flow. The final models, which include only significant terms, are shown below:

$$\begin{aligned} \text{Consistency component} = & 13.95 - 2.3 \times \text{span} - 23.79 \times \text{TSP} - 0.22 \times \text{DM} + \\ & 0.90 \times \text{TS} \times \text{DM} \quad (R^2 = 0.80) \end{aligned}$$

Equation 3.3

$$\begin{aligned} \text{Free-liquid flow} = & 9.96 - 0.015 \times d_{43} - 42.21 \times \text{TSP} - 0.30 \times \text{DM} + 0.13 \times \\ & \text{TSP} \times d_{43} + 0.000096 \times \text{DM} \times d_{43} + 1.11 \times \text{TSP} \times \text{DM} - 0.002 \times \\ & \text{TSP} \times \text{DM} \times d_{43} \quad (R^2 = 0.89) \end{aligned}$$

Equation 3.4

The model (Equation 3.3) indicated that consistency component was linearly correlated to distribution span, TSP, pectin DM and the interaction between TSP and pectin DM. Consistency component is inversely related to distribution span since no interaction term between span and the other two values was found. The extent of increase or decrease of consistency component at different TSP or pectin DM was different because of the interaction between these independent variables. The effect of TSP and pectin DM on consistency component is shown in Figure 3.7 when applesauce has a distribution span = 1.7. Increasing TSP causes an increase in consistency component when pectin DM is higher than 40%, but no significant effect is seen when pectin DM is less than 40% as there are enough available free methoxyl groups to bind with calcium to form a strong network. Overall, consistency component increased when sauce had lower distribution span and higher TSP, which could be achieved by using ripened apples with the cold break process. The effect of pectin DM on consistency component represented the variation found due to apple variety, and it was less affected by ripening and heat treatment.

Equation 3.4 shows that d_{43} , TSP, pectin DM and their interactions influenced free-liquid flow. Since there is a three-way interaction term, pectin DM was held constant to explain the change from other two variables, as it is an intrinsic factor depending mostly on variety. Figures 3.8a and 3.8b show a surface plot for free-liquid flow at pectin DM values of 40% and 55%, respectively. When the sauce has pectin DM = 40%, free-liquid flow decrease with d_{43} for all TSP levels. However, at higher pectin DM, the change in free-liquid flow depends on d_{43} and TSP. When sauce has pectin DM = 55%, free-liquid flow decreases with increasing TSP when d_{43} is more than 600 μm , or decreases if d_{43} is smaller when TSP is less than 0.30%. Applesauce with $d_{43} < 600 \mu\text{m}$ or high TSP $> 0.30\%$ has low free-liquid flow representing ideal values in the 0-0.5 cm range. Applesauce with low TSP and large d_{43} , will have high free-liquid flow, especially when pectin DM is higher than 40%. Therefore to achieve low free-liquid flow values, applesauce should be made from ripened apples and/or by the hot break process to achieve the targeted ranges for d_{43} and TSP.

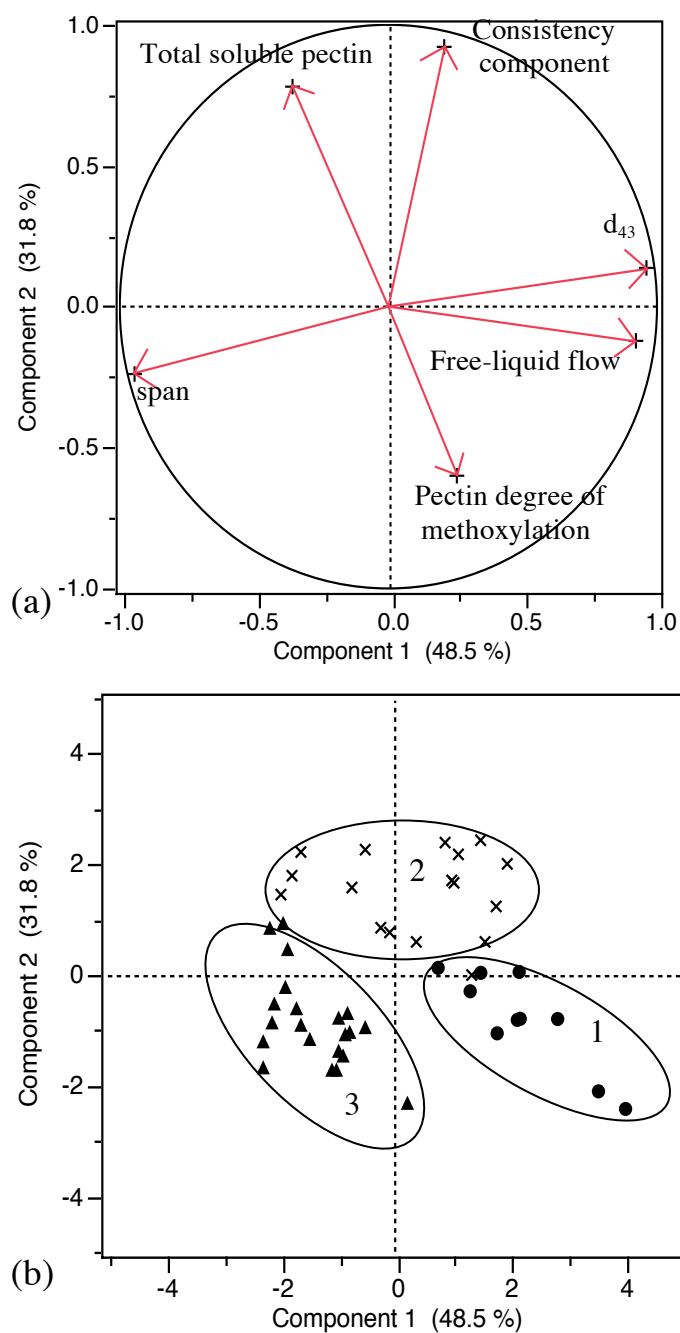


Figure 3.6 Principal component analysis of applesauce for rheological and physico-chemical properties in plane defined by first two components. (a) correlation plot (b) product map; ellipses represent samples grouped by hierarchical ascendant classification

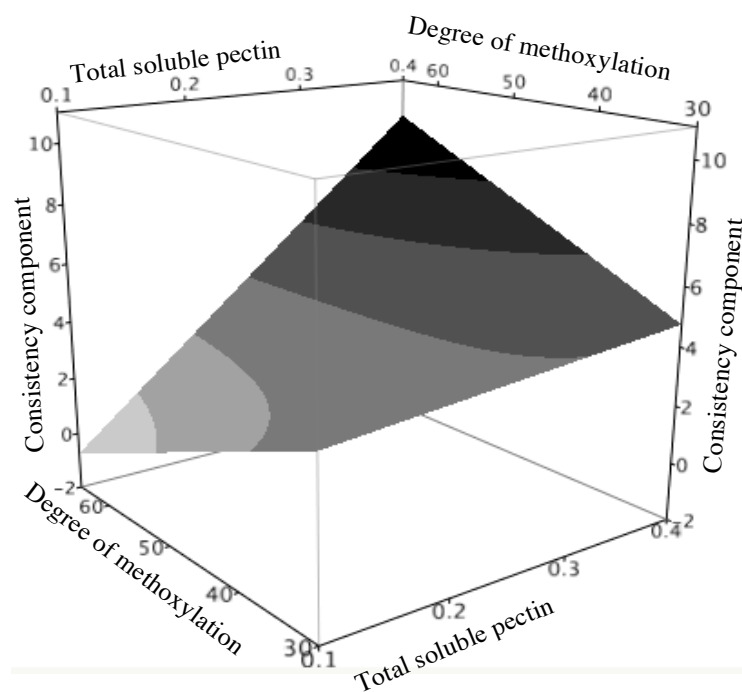


Figure 3.7 Response surface for the effect of total soluble pectin (g galacturonic acid equivalent per 100 g applesauce) and degree of methoxylation (%) on consistency component at distribution span equals to 1.7

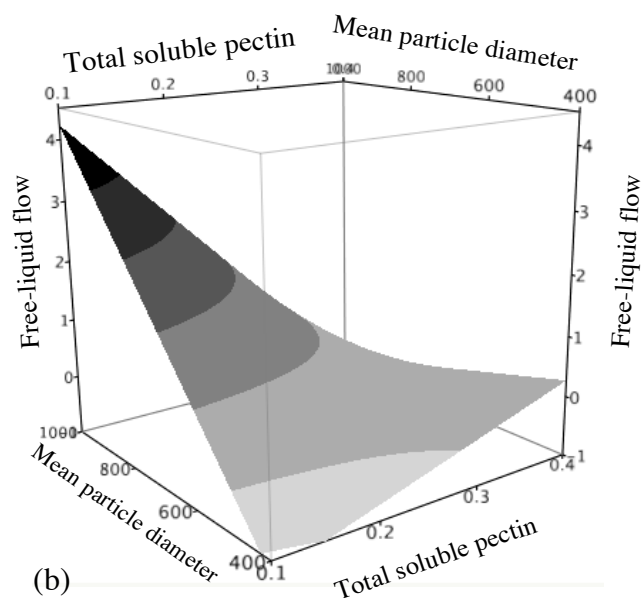
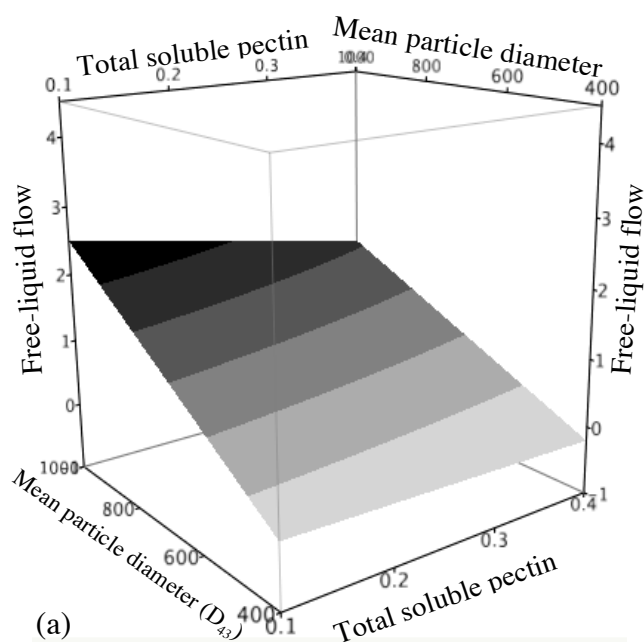


Figure 3.8 Response surface for the effect of total soluble pectin (g galacturonic acid equivalent per 100 g applesauce) and mean particle diameter on free liquid flow at (a) pectin DM = 40% (b) pectin DM = 55%

Conclusion

Applesauce consistency depends on apple variety, post-harvest ripening stage, and processing conditions (extractor speed and heat treatment effect), which are related to the differences in chemical compositions and physical properties. Controlled post-harvest ripening and heat treatment could be used to improve sauce quality (thicker sauce with less syneresis) by increasing total soluble pectin and by attaining targeted particle size distributions (smaller mean particle diameter and wider particle size distribution). Hot break sauce had consistent quality regardless of apple variety and ripening; therefore, the hot break process could be used to overcome the variation associated with these two factors. By understanding the changes in applesauce physico-chemical properties that are derived from fruit handling and processing treatments, and how they relate to rheological parameters (consistency component and free-liquid flow), applesauce producers could apply appropriate conditions to achieve targeted applesauce consistency with significant quality improvements.

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CHAPTER 4

PHYSICO-CHEMICAL AND RHEOLOGICAL PROPERTIES OF
APPLESAUCE: EFFECT OF DICED APPLE ADDITION TO COLD
BREAK SAUCE BEFORE COOKING AND FINISHING

Abstract

We studied the effect of adding diced apples to cold break applesauce prior to cooking and finishing on rheological and physicochemical properties of the final sauce. Apples (Crispin, Cortland, and Jonagold) were diced (0.64, 1.27, 1.90 cm) and added to extractor output, cold break sauce, at 0% to 75% ratio. Mixtures were cooked (90°C x 16 min), pulped and hot-packed. To further test the variety effect, 4 groups of blended varieties were studied at 0.64 cm dice size and 50% diced apple. Samples were analyzed for rheological, physical and chemical properties. Results were evaluated by ANOVA and Tukey's HSD test ($p \leq 0.05$). Sauce was thicker when processed from smaller dice size and/or higher % diced apple. For all dice sizes, the addition of diced apple into extractor output caused an increase in total soluble pectin and particle size distribution span, and a decrease in syneresis, mean particle diameter, and serum separation. The effect was pronounced on varieties producing thin sauce and/or high syneresis when made by the cold break process. Applesauce made with $\geq 50\%$ diced apple addition was significantly different from control (0%) for all dice sizes, and had consistent quality regardless of varietal effect. Therefore, the addition of diced apple prior to cooking and finishing could be used to improve sauce quality (thicker sauce and less syneresis) and overcome variation associated to variety.

Significant correlations were found between applesauce consistency and total soluble pectin ($r = 0.42$), and between serum separation with distribution span, mean particle diameter and total soluble pectin ($r = -0.92, 0.93, -0.53$, respectively).

Introduction

Applesauce is a typical American product, accounting for 30-40% of apple-processed products in 2000-2010 (USDA 2012). According to the product Identity Standards, applesauce is the food prepared from comminuted or chopped apples, which may have added thereto ingredients specified by the regulation (FDA 2012). From a physical point of view, it can be considered as a concentrated dispersion of insoluble matter (pulp) in an aqueous medium (serum), resulting from heat and mechanical treatment applied to the native fruits (Tarea and others 2007).

Viscosity has been traditionally one of the main quality attributes considered to determine overall quality and acceptability of applesauce products (Schijvens and others 1998). Previous work has shown that applesauce rheology is clearly influenced by processing variables, that is, finisher screen opening size, finisher speed and heating effect. Increasing finisher screen opening size and speed resulted in larger mean particle size (Nogueira and others 1985; Rao and others 1986; Schijvens and others 1998); however a significant increase in consistency index was observed only when increasing screen opening size (Nogueira and others 1985). Schijvens and others (1998) observed the importance of cooking time on applesauce apparent viscosity, which positively related to pectin degradation and pulp content. Godfrey and others (1995) found that blanching apples at 59-71°C before pulping result in thicker sauce.

Furthermore, it has been demonstrated that the influence of processing parameters on the rheological properties of applesauce dramatically depends on apple variety.

Traditionally, applesauce was processed by the hot break process. Apples were chopped and precooked before pulping. The object of precooking was to soften the fruit for subsequent pulping and to inactivate polyphenol oxidase to prevent enzymatic browning (Wiley and Binkley 1989). Nowadays, industrial applesauce production has changed from hot break to cold break process due to the utilization of a machine, a turbo extractor, which could pulp the whole fruit to sauce or puree at room temperature without any thermal pretreatment. However, this could lead to the production of thin sauce with free liquid separation, depending on apple varieties and ripening stages. Applesauce with low viscosity also caused problems at the filling step as it can overflow the primary package prior to capping or sealing, resulting in considerable financial losses for the industry.

Heat treatments could modify the activity of cell wall degrading enzymes and consequently affect the modification of cell wall components and result in structural changes (Lurie 1998). Kunzek and others (1999) also reported that heat treatments could influence the physico-chemical and especially the hydration properties of cell wall materials. Hot break sauce had less syneresis than from cold break sauce, and a similar result was found in tomato puree processing where preheating tomatoes to 77-93°C before extraction increased viscosity and reduced syneresis (Verlent and others 2006). However, due to the growing interest in efficient food processing procedures that reduce heat input and steam consumption, and that better retains flavor, color, and nutritional value, applesauce processed from cold break process is preferred.

Therefore, to balance product quality and energy consumption, applesauce could be processed utilizing an optimized procedure that applies both cold and hot break processes.

Our group assessed the influence of the addition of diced apples into extractor sauce output (cold break) before cooking and finishing (hot break) on rheological, physical and chemical properties of varietal applesauce. We evaluated the effect of apple dice size and the proportion added to extractor output to determine the optimal conditions to improve sauce quality. To resemble industrial practices, we also evaluated the effect of diced apple addition utilizing a blend of varieties on applesauce attributes.

Material and Methods

Materials and processing methods

Three apple varieties (*Malus domestica* Borkh.), Crispin, Cortland, and Jonagold, were sourced from local, upstate New York farms, 2011 harvest, and were placed in cold storage at 1°C, 95% relative humidity for 4 months to resemble commercial storage prior to processing. Apples were processed into applesauce following the procedure described in Figure 4.1. Apples were diced by a Dicer (Model No.C, Urschel Laboratories Inc., Valparasio, IN) into 0.64, 1.27, and 1.90 cm dices and mixed with extractor output (cold break sauce) from a Turbo extractor (Bertocchi CX5, Bertocchi SLR, Parma, Italy) with screen opening size of 1.6 mm and rotation speed of 1800 rpm at four different ratios: 0%, 25%, 50% and 75%. A small amount of

10% ascorbic acid solution was added to prevent browning. Water (15%) was added to the mixture to standardize soluble solids and heated in an agitated steam kettle (Groen MFG. Co., Chicago, IL) at 95°C for 14 min. The mixtures were pulped to applesauce by feeding to a finisher with screen opening size of 3.2 mm and rotation speed of 500 rpm to remove skin and seeds from diced apples. Applesauce was hot-filled into 16 oz glass jars, capped and immediately turned upside down for 2 min for cap sterilization, and finally cooled in a water bath to room temperature.

To resemble industrial practices, the effect of blended varieties on diced apple addition was evaluated. Apples were sourced from upstate New York farms, 2011 harvest, and kept in controlled atmosphere storage (-1-2°C, 1-3 % O₂, 4% CO₂, (Hardenburg and others 1986)) for 6-8 months before delivered to the New York State Agricultural Experimental Station (Geneva, NY). After receiving, apples were placed in cold storage at 1 °C and 95% relative humidity and processed within 2 weeks into applesauce following procedures described in Figure 4.1. Apples were diced (0.64 cm) and mixed with extractor output (cold break sauce) at 0% (control) and 50% diced apple. Preliminary analysis of six individual variety applesauces allowed us to categorize them into four groups, based on similar sauce quality: 1) 50% Crispin and 50% Jonagold (B1) – challenging varieties; 2) 50% Idared and 50 % Golden Delicious (B2) – challenging varieties; 3) 50% Cortland and 50% McIntosh (B3) – good varieties; 4) all six varieties (B4).

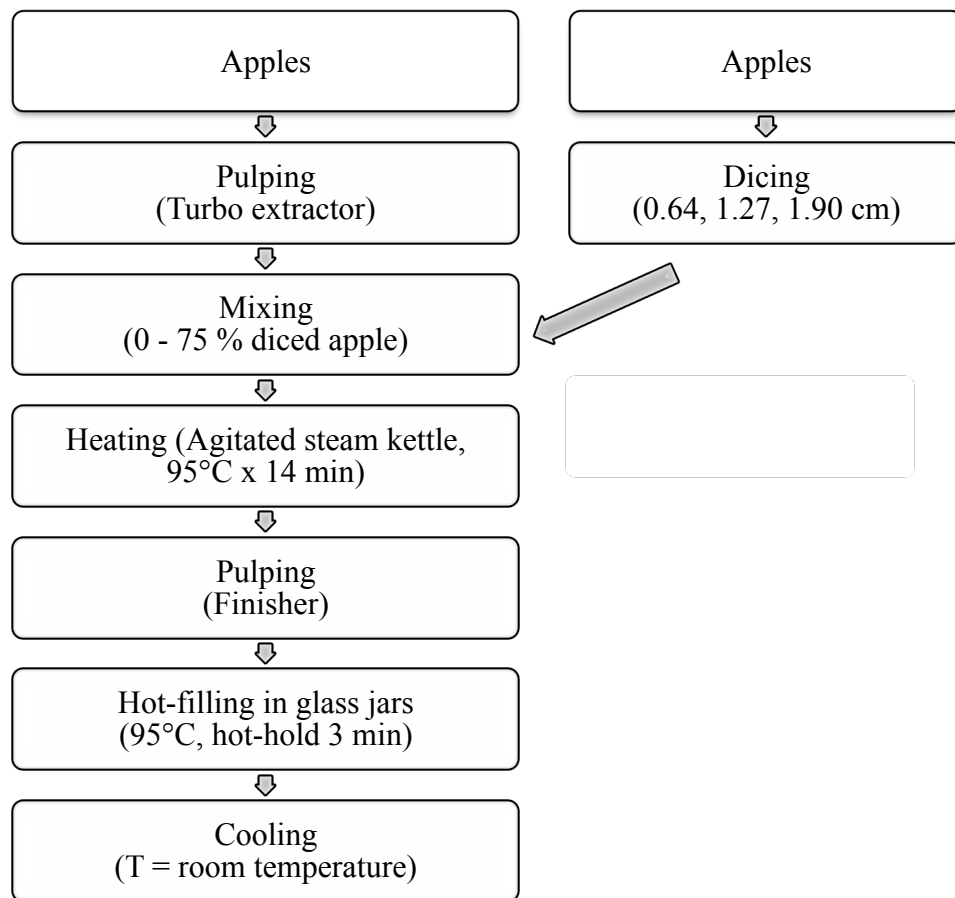


Figure 4.1 Flow chart of applesauce processing with diced apple addition

Rheology measurements

The measurements were carried out on a Brookfield DV-III Ultra programmable rheometer (Brookfield Engineering Laboratories, INC. Middleboro, MA) with V73 plus spindle. The measurements were determined by applying shear rate from 0.5 to 3.0 s⁻¹ and back at 25°C during a total time of 11 minutes, and taking readings every minute. Then the consistency index and yield stress were determined for each sample using Power law Model and Casson's Model. Based on the Grading Manual for Canned applesauce (USDA 2005), USDA consistency of sauce and liquid

were measured at room temperature using the USDA Consistometer flow sheet no.1, by averaging sauce and liquid spread of products subjected to flow for 1 minute on a plastic chart containing concentric circular markings, 0.5 cm apart. USDA consistency free-liquid is achieved by subtracting sauce from liquid flow.

Particle size measurements

Particle size distribution measurements were performed using laser diffraction (Mastersizer model MS-2000, Malvern Instrument Inc., Westborough, MA). The volume based (d_{43}) diameters and the width of distribution (distribution span) were obtained for every sample:

$$d_{43} = \sum_i n_i d_i^4 / \sum_i n_i d_i^3 \quad \text{Equation 4.1}$$

where n_i is the number of particles of diameter d_i .

$$\text{Distribution span} = (d_{90\text{th percentile}} - d_{10\text{th percentile}}) / d_{50\text{th percentile}} \quad \text{Equation 4.2}$$

pH, total soluble solids, total solid

Applesauce samples were measured for pH using a pH meter (Accumet Basic AB15 pH meter; Fisher Scientific Waltham, MA), soluble solids with a Leica Auto ABBE refractometer (Leica Inc., Buffalo, NY) and reported as °Brix, and total solids content obtained according to AOAC (2000).

Serum separation

Serum separation was determined according to the methods of Daou and Zhang (2011) with some modifications. Sample (~50 g) was weighed and centrifuged

(3620 x g, 5000 rpm) for 5 minute and applesauce serum was collected. Serum separation was expressed as the percentage (w/w) of the amount of water released from the sample.

Total soluble pectin and pectin degree of methoxylation

Alcohol insoluble residue was determined following procedures described by Colin-Henrion and others (2009). Soluble pectin content (as galacturonic acid equivalent) was determined according to Fraeye and others (2009) and pectin degree of methoxylation was determined by using alcohol oxidase and Purpald as described by Anthon and Barrett (2004) in water-soluble and chelator-soluble pectin fractions of applesauce, which were proportionally combined as fractions of total soluble pectin and overall pectin degree of methylation of sauces.

Statistical Analysis

Two or three apple batches were processed for each processing treatment and two jars of applesauce were collected for each batch. Measurements for all experimental units were conducted in duplicate or triplicate. Statistical analysis was performed using JMP 10.0 (SAS Institute Inc., Cary, NC). Before running the ANOVA, we tested for potential multicollinearity and performed Principal Component Analysis (PCA) for rheological properties, and chose the number of components so that 90% of variability is explained. To test differences between main effects of dice size, and % diced apple, we used Tukey's Honestly Significant Difference (HSD) at 95 % confident interval. Correlations between variables were

tested with the Pearson correlation. For the effect of blended variety, PCA was analyzed to calculate multivariate product spaces to examine all variable effects and hierarchical ascendant classification (HAC) was performed.

Results and Discussion

Effect of diced apple addition on rheological properties

Applesauce samples had soluble solids (10-14 °Brix) and pH (3.3 – 3.7) values in agreement with previous literature (La Belle 1981). All applesauces were shear-thinning ($n < 1$), and consistency index and yield stress (70 – 100 Pa.s, and 62 – 93 Pa) were comparable to those previously reported in the literature (7 – 50 Pa.s, and 31 – 87 Pa) (Rao and others 1986; Qiu and Rao 1988; Schijvens and others 1998). USDA consistency sauce (3.5-4.6 cm) and free-liquid (0-2 cm) were similar to a previous study by our group.

Since applesauce consistency index was highly correlated to yield stress and USDA consistency sauce ($r = 0.79, -0.80$, respectively), PCA was performed to increase the power of analysis. Applesauce rheological properties were described by the first two components, which accounted for 66% and 26% of the total sum of squares and kept for analysis (Table 4.1). The first two components were named consistency component and free-liquid flow. The desired applesauce quality should have high consistency component (thicker sauce) and low free-liquid flow (less syneresis).

Table 4.1 Eigenvectors of principle component analysis of applesauce rheological properties by first two components

Parameters	Consistency component	Free-liquid flow
Power law consistency index	0.60	-0.02
Casson's yield stress	0.58	0.25
1/(USDA consistency sauce ²)	0.54	-0.02
USDA consistency free-liquid	-0.13	0.97

Dice size, % diced apple and their interaction had significant effects on consistency component ($p < 0.05$), and had similar trends for all varieties. Cortland applesauce had the highest consistency component, followed by Crispin and Jonagold. Increasing % diced apple addition to extractor sauce output significantly increased the consistency component only for 0.64 cm dice size (10-15% higher consistency index and yield stress, and 5-15% lower USDA consistency sauce) (Figure 4.2). In addition, sauce processed from 0.64 cm dice size had the highest consistency component among sauce made from the same % diced apple. Smaller diced apples had more surface area and shorter distance for the heat to reach the apple core, thus they were softer and easier to push through the finisher screen by the centrifugal force applied (McLellan 2005), resulting in applesauce with higher total solids. We found that total solid content increased with diced apple addition and was positively correlated with the consistency component ($r = 0.39$), which is in agreement with several studies on tomato sauces and purees (Tsai and Zammouri 1988; Yoo and Rao 1994).

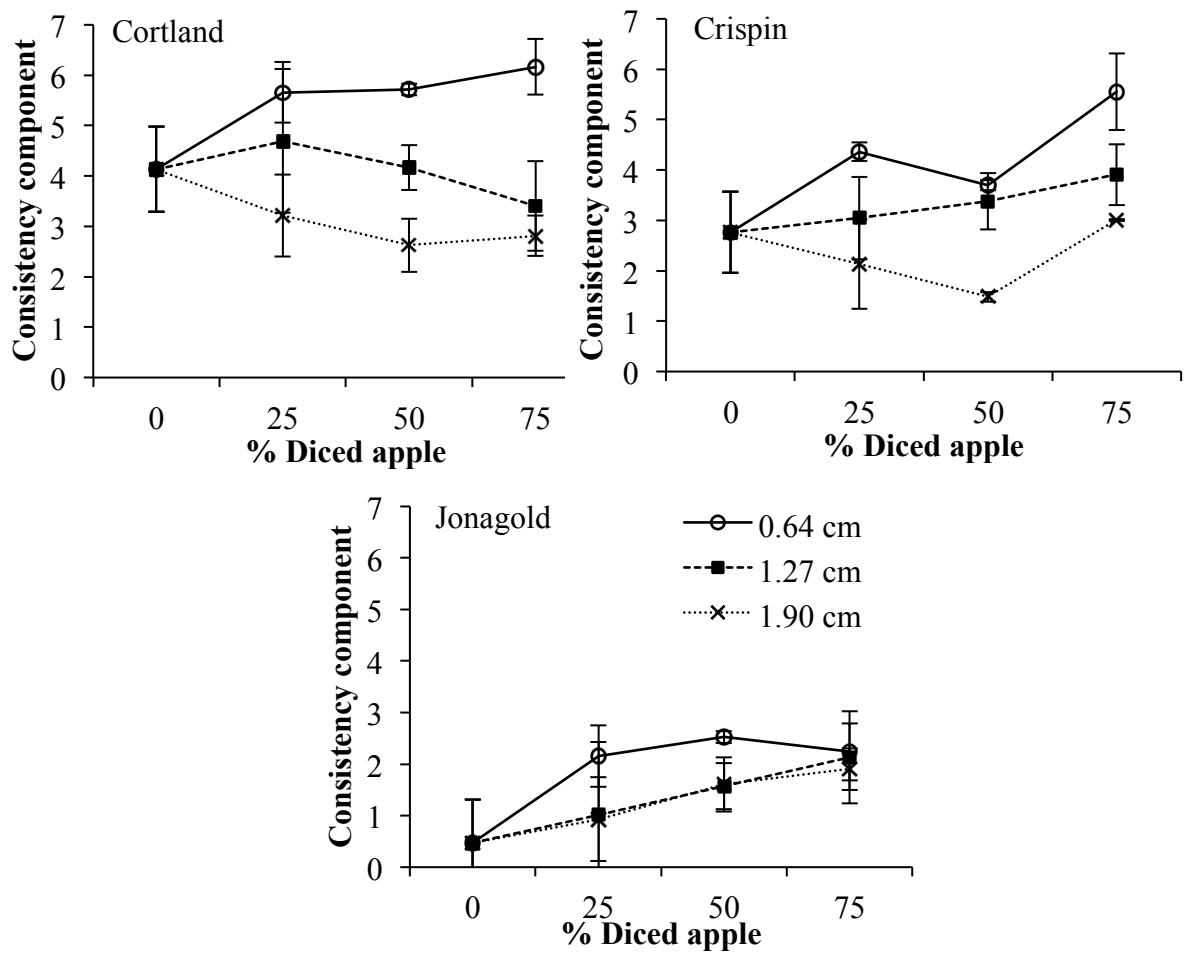


Figure 4.2 Effect of dice size and proportion of diced apple addition into extractor sauce output on consistency component (dimensionless) of varietal applesauce

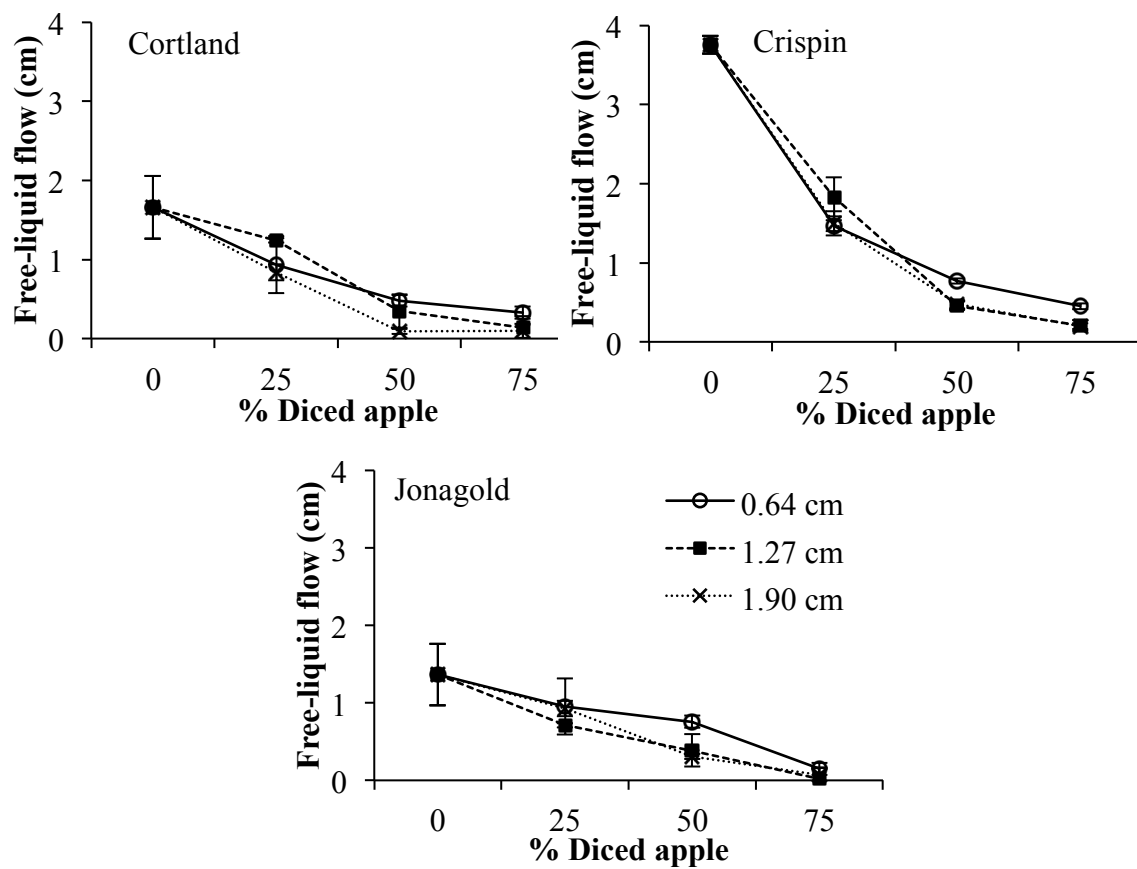


Figure 4.3 Effect of size and proportion of diced apple addition into extractor sauce output on free-liquid flow of varietal applesauce

Free liquid flow was significantly affected by variety, % diced apple and their interaction ($p < 0.0001$), but no effect from dice size was found. Crispin applesauce had the highest free-liquid flow, followed by Jonagold and Cortland. For all three varieties, free-liquid flow decreased with % diced apple (Figure 4.3). Applesauce processed from at least 50% diced apple was significantly different from 0% (control) on free-liquid flow, while variety had no effect. This indicated that at least 50% diced apple addition could overcome variations associated with variety and improve sauce quality (USDA consistency free-liquid < 0.7 cm). Since diced apples were cooked before final pulping, the tissue was softened as a result of changes in cell wall composition leading to weaker cell adhesion, causing cell separation instead of cell wall rupture during extraction (Waldron and others 1997). Thus, the higher the diced apple ratio added to extractor sauce output, the more cells separating in intact clusters than across the cells, causing less liquid release.

Effect of diced apple addition on serum separation

Serum separation (SS) showed the amount of liquid released from samples, which inversely represented water-binding capacity (WBC). WBC describes the ability of a sample to bind water when exposed to an external stress such as centrifugation or filtration (Kunzek and others 1999). SS was significantly influenced by variety and % diced apple ($p < 0.05$) but not by dice size, showing similar trends found on free-liquid flow. SS was positively correlated with free-liquid flow ($r = 0.79$) and significantly decreased (20-60%) with diced apple addition, showing better WBC. An increase in WBC may result from the modification in particle size distribution and

chemical properties, which will be discussed next. Since SS and % diced apple have a strong linear correlation, applesauce manufacturers could use Equation 4.3 to determine the appropriate % diced apple needed to achieve acceptable SS.

$$\text{Serum separation (\%)} = 57.2 - 0.4 \times \% \text{diced apple} \quad (R^2 = 0.93) \quad \text{Equation 4.3}$$

Effect of diced apple addition on particle size distribution

Apple variety, % diced apple and their interaction had significant effects on both distribution span and mean particle diameter (d_{43}) ($p < 0.05$). Distribution span and d_{43} ranged 1.6 - 2.4 and 500 - 860 μm , respectively. As there was no effect of dice size, Figure 4.4 presents only the change in particle size distribution for applesauce made from 0% to 75% diced apple addition (0.64 cm dice size), showing that distribution span (45-70%) increased and d_{43} (30-40%) decreased significantly with increments in diced apple proportion. As a general rule, a decrease in particle size yields a larger interfacial area, which results in stronger interparticle interactions and consequently higher viscosity values (Yoo and Rao 1994). However, the ‘Farris effect’ states that the consequence of broadening particle size distribution at a constant volume fraction is to reduce the viscosity of the dispersion because of higher maximum packing fraction (Ferguson and Kemblowski 1991). As a result, two opposite variables are influencing the rheological results obtained. This could explain why the consistency component had low correlations with distribution span ($r = 0.19$) and d_{43} ($r = -0.12$).

Free-liquid flow and serum separation, representing WBC, were negatively correlated to distribution span ($r = -0.73$ and -0.92 , respectively) and positively correlated to d_{43} ($r = 0.78$ and 0.93 , respectively). Similar results were observed for artichoke fiber (Lopez 1996), apple cell wall material (Vetter and Kunzek 2002), carrots, and potatoes (Bengtsson and others 2011), indicating that thermal pretreatment resulted in smaller particle diameter and higher WBC. According to Ferguson and Kemblowski (1991) and Do and others (2007), increasing particle size distribution width for a given solids volume fraction would increase the maximum volume fraction; therefore, there would be more voids between particles to uptake and bind with water.

Effect of diced apple addition on chemical properties

Alcohol insoluble residue (AIR) represents the sum of soluble and insoluble fiber (Mueller and Kunzek 1998). AIR in applesauce samples varied from 1.88% to 2.53 % and the range of results was comparable to those found in the literature for apples and applesauce (1.76-5.48%) (Fischer and others 1994; Colin-Henrion and others 2009). No effect of diced apple addition on AIR was found, showing no modification on total fiber. However, AIR was significantly dependent on variety; Cortland had the highest values, followed by Crispin and Jonagold.

The total soluble pectin contents (TSP) of applesauces are given in Figure 4.5, which varied significantly by apple variety. The results obtained from the control samples (0% diced apple) corresponded well to previous studies (Scalzo and others 2005; Vanoli and others 2009; Le Bourvellec and others 2011). Diced apple addition

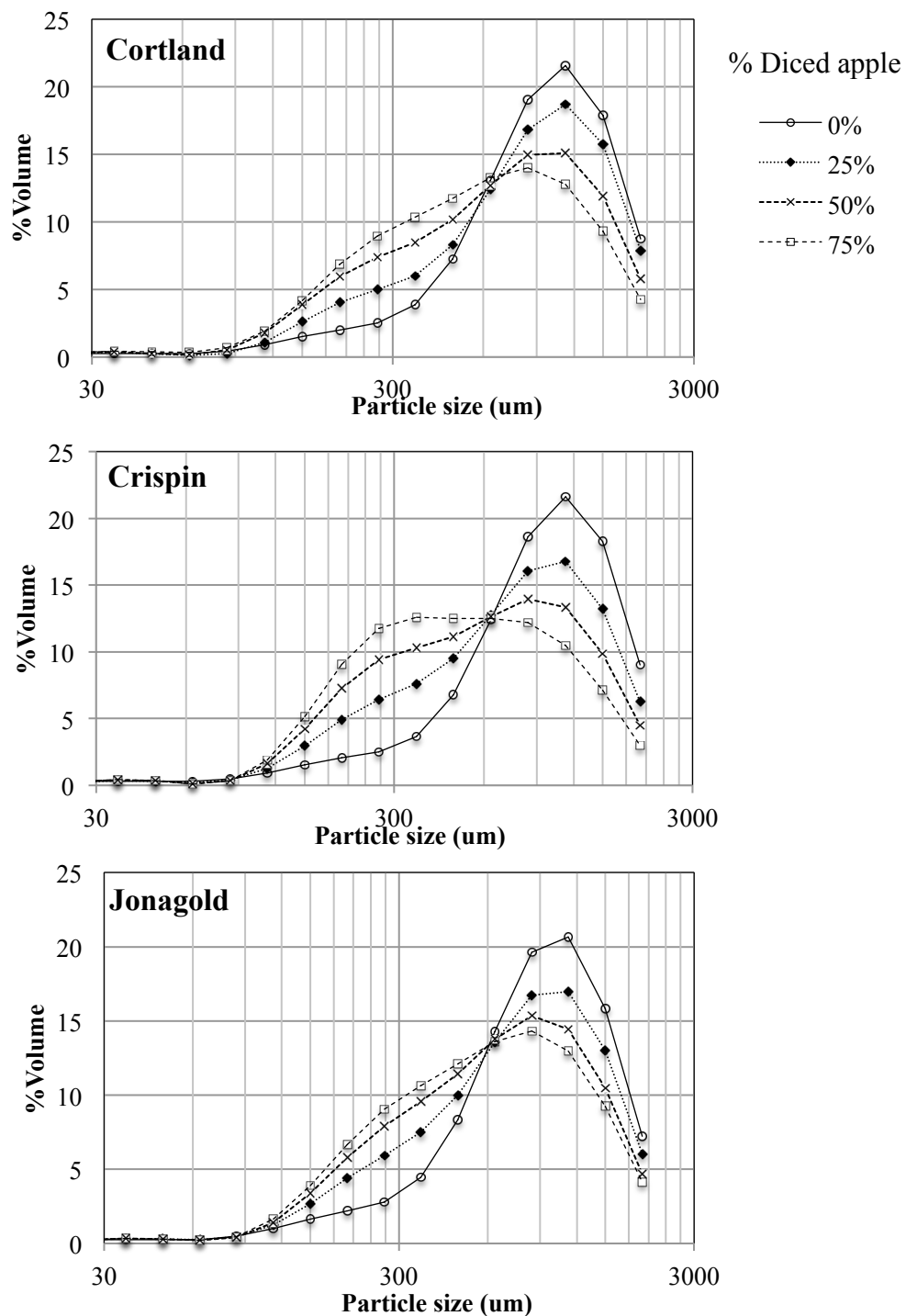


Figure 4.4 Effect of the addition of diced apple (0 – 75 %) at 0.64 cm dice size into extractor sauce output on particle size distribution of varietal applesauce

increased TSP for all apple varieties (25-70% increment), but there was no effect of dice size. TSP in applesauce with 50% or more diced apple was significantly different from sauce with 0% diced apple. An increase in TSP with diced apple addition was caused by the heating of the mixture of diced apple and extractor sauce output before final pulping or finishing. The thermal processing can change pectin solubility through β -elimination, resulting in an increase in soluble pectin content (Sila and others 2009). Our results were similar to previous studies on carrot, potato and apple fiber suspension (Kunzek and others 1999; Colin-Henrion 2009; Bengtsson and others 2011). Pectin is known to be a principal component for the formation of gels (Sila and others 2009); therefore increasing TSP could lead to a stronger gel structure or higher consistency. Additionally, a larger number of available hydrophilic groups such as hydroxyl and carboxyl from TSP could generally increase the binding of water close to the matrix, leading to better WBC (Einhorn-Stoll and others 2012). In this study, TSP was correlated with the consistency component ($r = 0.42$), free-liquid flow ($r = -0.56$) and serum separation ($r = -0.53$).

Pectin DM of total soluble pectin differed considerably between the varieties studied but was not affected by diced apple addition. Crispin had the highest pectin DM (70-88%), followed by Cortland (50-62%) and Jonagold (50-60%). Our results were comparable to those reported in the literature (47-88%) for apple varieties (Johnston and others 2002; Anthon and Barret 2008; Rascón-Chu 2009; Le Bourvellec and others 2011). Pectin DM was a significant factor for free-liquid flow and serum separation with $r = 0.58$ and 0.30 , respectively. Low pectin DM had better WBC

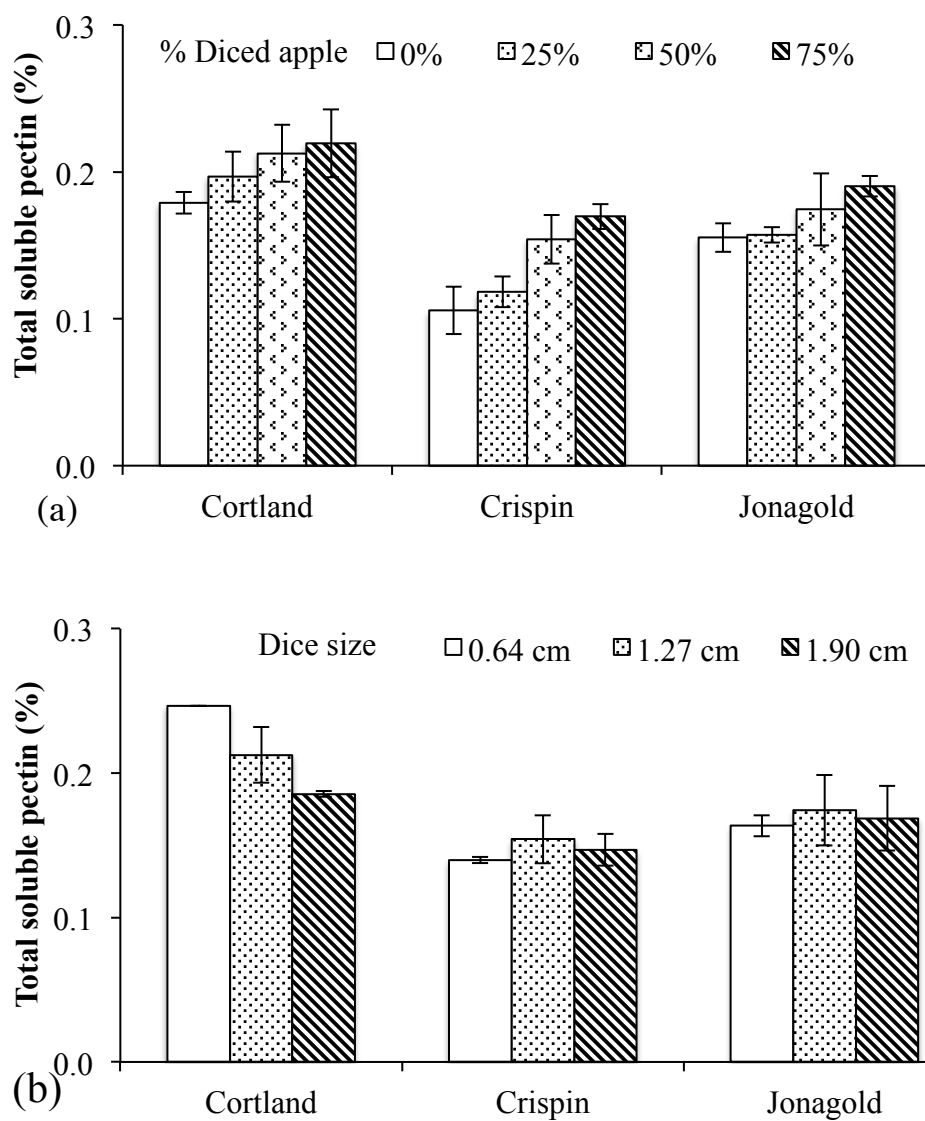


Figure 4.5 Effect of (a) % diced apple (0 -75 %) at 1.27 cm dice size and (b) dice sizes (0.64, 1.27, 1.90 cm) at 50 % diced apple on total soluble pectin content (g galacturonic acid equivalent per 100 g applesauce) of varietal applesauce

because of more available carboxyl groups to bind with water (Ping and others 2001; Einhorn-Stoll and others 2012).

Influence of blended varieties on diced apple addition effect

Blending varieties had significant impact on diced apple addition effect on sauce properties ($p < 0.05$). Due to high correlations in consistency index, yield stress and USDA consistency sauce, as well as, USDA consistency free-liquid and serum separation, only consistency index and USDA consistency free-liquid results are presented in Figure 4.6. At 0% diced apple, applesauce made from B3 (varieties that make thick sauce with minimum free liquid) and B4 (mix of all 6 varieties) had significantly higher consistency index than sauce made from B1 and B2, varieties that individually produce thinner sauce with more liquid separation. Interestingly, sauce made from B4 had a similar consistency to sauce made from B3, suggesting that applesauce processors could mix one-third of ‘good sauce’ varieties with two-third of challenging varieties to improve sauce consistency.

Compared within the same variety blend, sauce with 50% diced apple addition showed significantly higher consistency index for B1 and B2, while no significant changes were found for B3 and B4 (thicker sauce). However, it caused a significant decrease in USDA consistency free-liquid for all four blends. This showed that diced apple addition could increase sauce viscosity only when using apple varieties that produce thin sauce, but improve WBC for all apple blends studied. Thus, applesauce processors could use these findings as strategies to improve sauce consistency when they have to make sauce from challenging varieties, by adding $\geq 50\%$ diced apple,

and/or by mixing at least one-third of ‘good sauce’ varieties in the blend. The effect of diced apple addition on consistency index and USDA consistency free-liquid in relation to changes in physical and chemical properties, as discussed previously, and the correlation among these values are shown in Figure 4.7a. Sauce made from 50% diced apple (thick sauce with less syneresis) had smaller mean particle diameter, wider particle size distribution, and higher total soluble pectin.

The HAC performed on the PCA data outlined three groups of applesauce products (shown by ellipses in Figure 7b). The first group consisted of applesauce processed from B1 and B2 at 0% diced apple, having the thinnest sauce and lowest WBC. The second group is applesauce processed from B3 and B4 at 0% diced apple. This cluster is described as having thicker sauce than the first group but still having low WBC. Lastly, the third group was composed of all sauce processed from 50% diced apple, having the thickest sauce and highest WBC, showing best sauce quality. This showed that processing applesauce with 50% diced apple could improve sauce quality as well as overcome variations associated from apple variety.

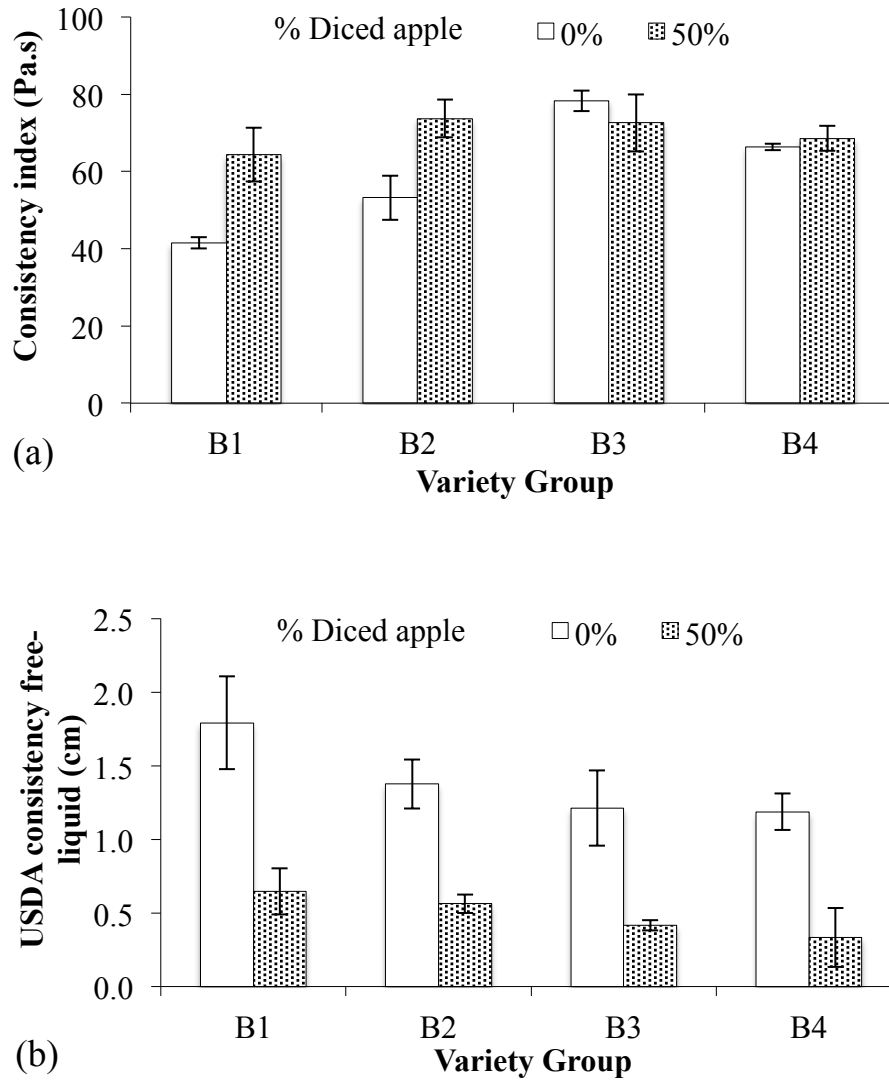


Figure 4.6 Effect of diced apple addition at 0% and 50% into extractor sauce output on applesauce made from four different blended varieties (B1 – Crispin & Jonagold, B2 – Idared & Golden Delicious, B3 – Cortland & McIntosh, B4 - All six varieties) on (a) consistency index and (b) USDA consistency free-liquid

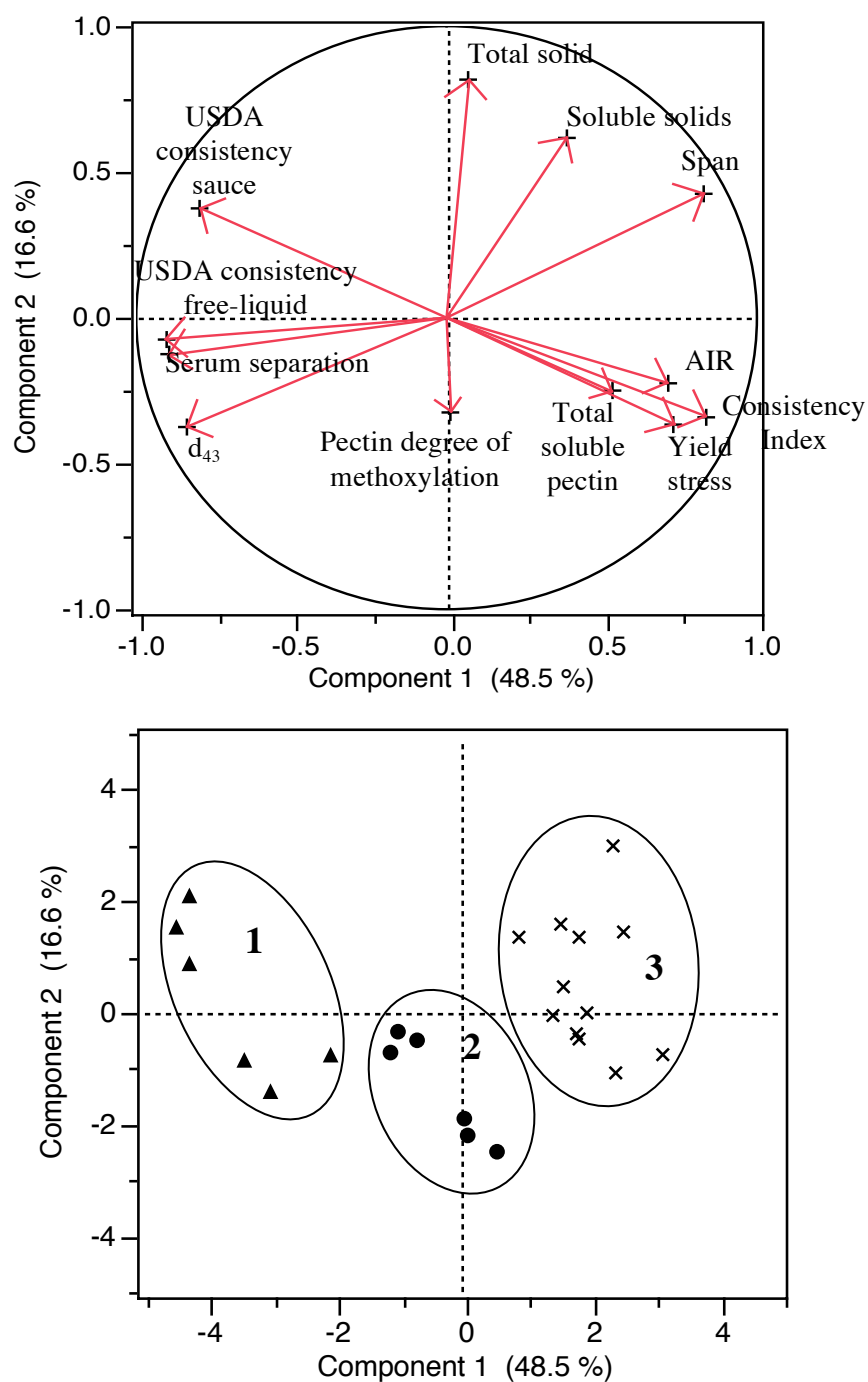


Figure 4.7 Principal component analysis of applesauce for physico-chemical and rheological properties in plane defined by first two principal components. (a) Product map; ellipses represent samples grouped by hierarchical ascendant classification. (b) Correlation plot

Conclusion

The addition of at least 50% diced apple into extractor sauce output before subjected to cooking and final pulping resulted in better applesauce quality (thicker sauce and less syneresis) due to the modification of particle size and particle size distribution, and increases in total solids and soluble pectin content. Diced apple addition had more effect on sauce processed from challenging varieties – those producing sauce with low viscosity and high syneresis. These findings are useful to applesauce producers that want to achieve consistent sauce quality regardless of varieties used, and could lead to the establishment of additional control measures to ensure targeted results.

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CHAPTER 5

EFFECT OF EXOGENOUS AND ENDOGENOUS PECTIN METHYL
ESTERASE ON PECTIN DEGREE OF METHOXYLATION AND ITS
IMPACT ON RHEOLOGICAL PROPERTIES OF APPLESAUCE

Abstract

We investigated the activity of exogenous and endogenous pectin methyl esterase (PME) on pectin degree of methoxylation (DM) and its impact on applesauce rheological properties. Three different varieties of apples (Crispin, Cortland, Jonagold) at 3 post-harvest ripening stages were pulped to applesauce, which was then treated with exogenous PME and/or calcium chloride, held at 20°C for 10-30 min before heating to 90°C, and hot-packed. To determine the effect of endogenous PME, a blend of two apple varieties (Crispin and Jonagold) consisting of 50% turbo extractor sauce output and 50% diced apple (0.64 cm size) was held at 55-71°C for 5-30 min to activate the PME; heated at 90°C, passed through a finisher and hot-packed. Samples were analyzed for rheological, physical and chemical properties. Results were evaluated by ANOVA and Tukey's HSD test ($p \leq 0.05$). Exogenous PME effect on applesauce properties was dependent on apple variety and more pronounced when using ripened apples. When PME was added to applesauce, the DM of water-soluble pectin (WSP, 60-87%) and chelator soluble pectin (CSP, 40-75%) decreased to 3-30%, and the galacturonic acid content in CSP increased. The decrease in DM by exogenous PME resulted in increased applesauce viscosity, especially when combined with Ca^{2+} , and reduced syneresis. Yet, high liquid separation was perceived with

PME+Ca²⁺ treatment. Additionally, we found that endogenous PME was activated at 55-60°C with 10 min holding time, inducing the decrease in DM of WSP from 83% to 60-70%. However, no changes in applesauce viscosity and syneresis were observed.

Introduction

In the U.S., apples are the second most consumed fruit following oranges (U.S. Apple 2011). In 2010, 4.2 million tons of apples, valued at \$2.2 million were consumed as fresh (68%) and processed products (32%). The top two processed products were apple juice and cider, and canned apple products such as applesauce (USDA 2012). Applesauce could be considered as a network of pectin molecules maintained together, or a dispersion of pulp, containing insoluble matters such as cell walls, in an aqueous medium (serum), containing pectin polysaccharides and other soluble substances (Lopez-Sanchez and others 2011). Applesauce is produced year round and changes in viscosity observed over the processing year are related to biochemical conversions of pectin, as a result of post-harvest fruit ripening during cold storage, and due to processing conditions.

Pectin is found in the plant middle lamella, playing a crucial role in cell-cell adhesion, and occurs in both the liquid and dispersed phase of plant-food dispersions due to its solubility characteristics (Van Buren 1979). Hence, the role of pectin on puree consistency and syneresis should not be neglected. One of the most abundant polymers in pectin is homogalacturonan (HG), a linear chain of galacturonic acid residues in which some of the C-6 carboxyl groups are methyl-esterified (Van Buggenhout and others 2009). The degree of methoxylation (DM) of HG is an

important factor for the pectin functionality in fruit and vegetables products. Pectin demethoxylation can improve the adhesion since the increase in free pectic carboxyl groups provides a greater opportunity for pectic polymers to be cross-linked with divalent ions such as Ca^{2+} (Christiaens and others 2012b). This can be achieved by the application of exogenous PME. The vacuum treatment with a PME solution containing Ca^{2+} significantly increased the firmness of fruits like canned half peaches (Javeri and others 1991), pasteurized apple cubes, strawberry halves and raspberries (Degraeve and others 2003), and diced tomatoes (Anthon and others 2005). The reduction in DM also decreases the rate and the extent of β -eliminative degradation at high temperature; however, lowering the DM of pectin may increase its sensitivity to acid hydrolysis (Sila and others 2006; Fraeye and others 2007).

A decrease in the pectin DM can also be achieved by activating endogenous PME. It has been long known that preheating vegetables to above 50°C , in a so-called low-temperature blanch, activates PME and results in a firmer final product through subsequent high-temperature processing. This effect has been extensively studied and shown to occur in a number of vegetables (Van Buren 1979; Ng and Waldron 1997; Anthon and others 2005; Ni and others 2005). Godfrey and others (1995) found that viscosity of applesauce increased by blanching apples at $59\text{--}71^{\circ}\text{C}$ for about 20 min before cooking and pulping, and believed that it was the result from endogenous PME activity.

Many studies have been carried out on the rheological properties of applesauce, but they are often related to parameters such as particle size, pulp content, and total soluble solids (Rao and others 1986; Qiu and Rao 1988; Yoo and Rao 1994;

Godfrey and others 1995). Detailed research towards the role of pectin on the flow properties of applesauce and the effect of PME on applesauce is lacking. It is known however that pectin structural modifications during processing remarkably alter the textural/rheological properties of plant-based foods (Van Buggenhout and others 2009). The objective of this study was thus to explore the effect of both exogenous and endogenous PME on applesauce pectin DM as well as its influence on applesauce rheology, syneresis, particle size distribution, and chemical properties. Apple variety and post-harvest ripening stage effects were taken into consideration by using 3 different apple varieties at 3 different post-harvest ripening stages for the exogenous PME study and a blend of two varieties for the endogenous PME study.

Material and Methods

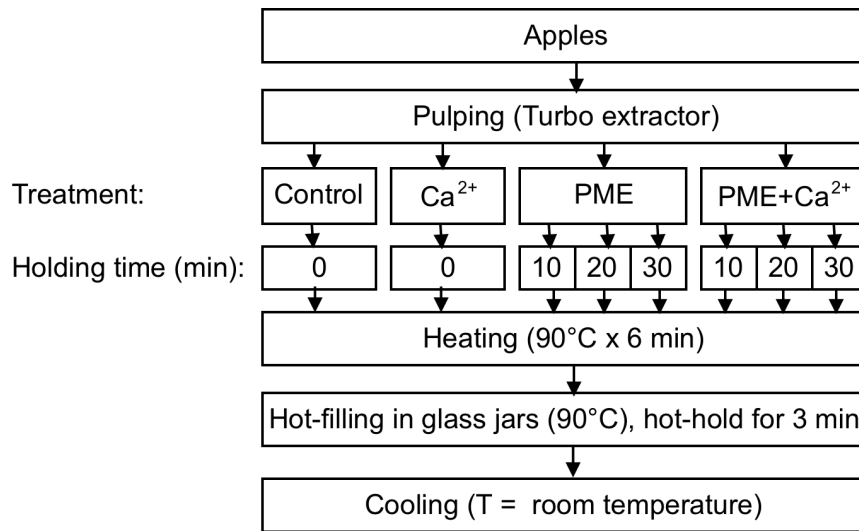
A schematic overview of the experimental set-up is presented in Figure 5.1. For the exogenous PME study, the enzyme treatment was applied to the sauce. For the endogenous PME study, a blend of sauce and diced apple was used to treat both the sauce and the apple tissue prior to pulping.

Exogenous pectin methyl esterase treatment

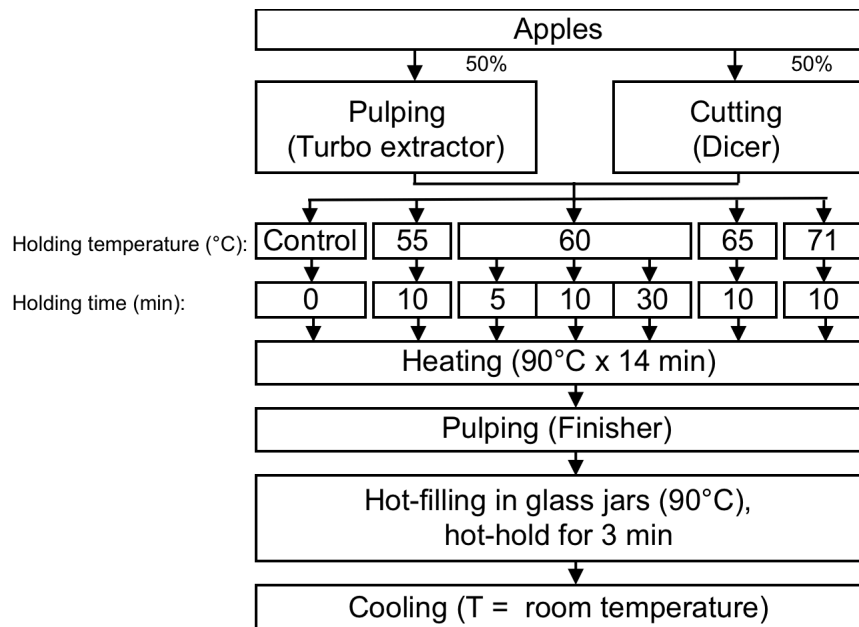
Preliminary study: we conducted a trial to test the effect of endogenous PME at 20°C. Four apple varieties (Jonagold, Golden Delicious, Empire, R.I. Greening) were pulped by a Turbo extractor and the sauce held at 20°C for 0 (control), 10 and 30 min, before heating to 90°C, and hot-filled to glass jars. Samples were tested for consistency index and USDA consistency values. Results showed no significant differences between

control (no holding) and treated sauce, indicating that endogenous PME activity was negligible at this temperature.

Exogenous PME study: three apples varieties (Crispin, Cortland, Jonagold) were stored at 10°C and 95% relative humidity immediately after harvest (between September and October of 2011). The effect of post-harvest fruit ripening stage was assessed by analyzing sauce made from fruit stored for 0, 20 and 40 days. Prior to processing, apples were weighed and tested for firmness using a hand-held penetrometer model FT 327 (Wagner Instruments, Greenwich, CT). Apples (~ 15 kg) were pulped by a Turbo extractor (Bertocchi CX5, Bertocchi SLR, Parma, Italy) with screen opening size of 1.6 mm rotating at 1800 rpm. Water (15% w/w) and a small amount of 10% ascorbic acid solutions were added to prevent browning. Four different treatment conditions were applied. One group did not undergo any treatment (control). The other three groups were treated with 200 ppm Ca^{2+} solution (Ca^{2+}), 250 ppm PME-solution (PME) or a solution containing both 250 ppm PME and 200 ppm Ca^{2+} (PME+ Ca^{2+}). PME and PME+ Ca^{2+} -treated sauce were held at 20°C for 10, 20, and 30 min. Calcium solution contained 0.5% (w/v) $\text{CaCl}_2 \cdot \text{H}_2\text{O}$ and PME solution was prepared using Rapidase® PEP due to its high activity at room temperature (obtained from *Aspergillus niger*, DSM Food Specialties USA, Inc., Charlotte, NC). Finally, applesauce was heated ($t = 6$ min, $T = 90^\circ\text{C}$) in an agitated steam kettle (Groen MFG. Co., Chicago, IL) and hot-filled in 16 oz glass jars. Jars were immediately turned upside down for 2 min for cap sterilization and cooled in a water bath to room temperature.



(a)



(b)

Figure 5.1 Schematic overview of the experimental set-up to evaluate the effect of pectin methyl esterase (PME) (a – exogenous and b – endogenous) on applesauce rheology and particle size distribution

Endogenous pectin methyl esterase treatment

Preliminary study: we first assessed the effect of endogenous PME on Jonagold applesauce. After pulping Jonagold apples using a Turbo extractor, sauce samples were held at 4 different temperatures (55, 60, 65, 71°C) for 0 (control) and 30 min.. Results showed no significant differences in USDA consistency and consistency index between control and treated sauce. As endogenous PME activation has been successfully applied to firm fruit pieces, we decided to conduct the main experiment by using a blend of sauce and diced apple.

Endogenous PME study: a blend of Crispin and Jonagold apples was used in this study. Apples were harvested in October of 2011 and stored in Controlled atmosphere (CA) storage conditions (-1-2°C, 1-3% O₂, 4% CO₂ - Hardenburg and others 1986) for 10 months prior to processing. Applesauce processing was similar to the method applied to the exogenous PME study except half of apples were diced (1.27 cm) by a Dicer Model No.C (Urschel Laboratories Inc., Valparasio, IN) and mixed with the extractor sauce output. The mixture was held at 55, 65, and 71°C for 10 min and at 60°C for 5, 10, and 30 min. There was no hold time or heating for control sauce. The mixture was then heated to 90°C for 14 min in an agitated steam kettle. In this study, sauce was pulped a second time by a finisher (screen opening size 3.2 mm, rotation speed 500 rpm) due to the addition of diced apple prior to hot-filling and cooling to room temperature.

Rheology measurements

The measurements were carried out on a Brookfield DV-III Ultra

programmable rheometer (Brookfield Engineering Laboratories, INC. Middleboro, MA) with V73 plus spindle to obtain values of shear stress for each applesauce sample every minute. The measurements were determined by applying shear rate from 0.5 to 3.0 s⁻¹ and back at 25°C during a total time of 11 min. Based on the Grading Manual for Canned applesauce (USDA 2005), USDA consistency sauce and liquid were measured at room temperature using the USDA Consistometer flow sheet no.1, by averaging sauce and liquid spread of products subjected to flow for 1 min on a plastic chart containing concentric circular markings, 0.5 cm apart. USDA consistency free-liquid was achieved by subtracting sauce from liquid flow. Applesauce serum was collected after centrifuging applesauce at 41,840 x g (17,000 rpm) for 30 min (Sorvell SA-600 Centrifuge; ThermoScientific, Waltham ,MA) and assessed for capillary viscosity using a Cannon glass capillary viscometer (model Cannon-Fenske routine, Cannon instrument company, State College, PA).

Particle size measurements

Particle size distribution (PSD) measurements were performed using laser diffraction (Mastersizer model MS-2000, Malvern Instrument Inc., Westborough, MA). The volume based (d_{43}) mean particle diameter and the width of distribution (span) were obtained:

$$d_{43} = \sum_i n_i d_i^4 / \sum_i n_i d_i^3 \text{ where } n_i \text{ is the number of particles of diameter } d_i$$

Equation 5.1

$$\text{Distribution span} = (d_{90\text{th percentile}} - d_{10\text{th percentile}}) / d_{50\text{th percentile}}$$

Equation 5.2

Chemical properties measurements

Applesauce was analyzed for pH, total soluble solids, and total solids. pH was measured with a pH meter (Accumet Basic AB15 pH meter; Fisher Scientific Waltham, MA). Total soluble solids was measured with a Leica Auto ABBE refractometer (Leica Inc., Buffalo, NY) and reported as °Brix. Total solids content was obtained according to AOAC (2000). Applesauce serum was analyzed for Ca^{2+} content using the Calcium-Arsenazo quantification kit (BEN Biochemical Enterprise, Milano, Italy)

Galacturonic acid content and pectin degree of methoxylation (DM) measurements

Alcohol insoluble residue (AIR) was determined following procedures described by Colin-Henrion and others (2009) and then fractionated by extracting with water and a chelating agent to obtain water-, and chelator-soluble pectin (CSP), respectively and analyzed for galacturonic acid content, as described by Fraeye and others (2009). The amount of methanol was determined by using alcohol oxidase and Purpald as described by Anthon and Barrett (2004) and pectin DM was calculated as the ratio of the molar amount of methanol esters to the molar amount of galacturonic acid.

Statistical Analysis

Two or three apple batches were processed for each processing treatment, and two jars of applesauce were collected for each batch. Measurement for all

experimental units was conducted in duplicate or triplicate and results were analyzed by ANOVA using JMP 10.0 (SAS Institute Inc., Cary, NC). To test differences between main effects, we used Tukey's Honestly Significant Difference (HSD) at 95 % confident interval.

Results and Discussions

Apple and applesauce characteristics after exogenous PME treatment

According to Johnson and others (2002), firmness can be used as an indicator for apple ripening. During fruit storage at 10°C and 95% relative humidity (RH) for up to 40 days, firmness significantly decreased with ripening days; 65-70 N at day 0, 42-54 N at day 20, and 40-45 N at day 40 for all three varieties. Therefore, processing at days 0, 20 and 40 is a reasonable representation of ripening changes that occur from freshly harvested to fully ripened apples.

The non-treated applesauce (control) had total soluble solids of 10-13 °Brix, total solids content of 12-15% and pH of 3.1-3.5, varying among apple variety and post-harvest ripening stage, in agreement with previous literature (La Belle 1981; Massey Jr. 1989). Overall, applesauce had higher pH and total soluble solids values with progress of fruit storage associated with fruit ripening, but the changes did not have an effect on the calcium and/or PME treatments.

Influence of exogenous PME on rheological properties of applesauce and applesauce serum

Rheological properties of applesauce were well explained with more than 90% confident of fit by the power law model with the parameters consistency index and flow behavior index (n), and by the Casson's model with the parameter yield stress, as shown in Table 5.1. Those two models have previously been used for the description of applesauce and apple puree (Rao and others 1986; Qiu and Rao 1988; Godfrey and others 1995; Cantu-Lozano and others 2000).

Flow curves of different treatment of applesauce at different ripening days are shown in Figure 5.2. All applesauce samples showed pseudoplastic behavior ($n < 1$) as apparent viscosity diminishes with the shear rate. Significant differences ($p < 0.001$) were observed in n -values of PME treated sauce, while fruit ripening over storage was not a significant factor for observed changes. The n values ranged from -0.02 to 0.08 for control and Ca^{2+} -treated sauce and ranged from -0.15 to 0.00 for PME and PME+ Ca^{2+} sauce. The decrease in n values led to a more shear-thinning behavior, relating to stronger interactions between the particles.

Treated applesauce viscosity was measured and compared to the corresponding control sauce (same apple variety and storage time). The ANOVA analysis showed that the exogenous PME treatment indeed had significant effects on applesauce rheological properties ($p < 0.001$), especially when PME treatment was combined with Ca^{2+} solution. The effect depended on apple variety and storage time: applesauce made from longer stored apples was more susceptible to PME activity than fresh ones, and Cortland applesauce was more affected than Crispin and Jonagold. Yet, no effect

Table 5.1 Consistency index, flow behavior index (n, dimensionless), yield stress and USDA consistency values of applesauce after different kinds of pectin methyl esterase (PME) and/or Ca²⁺ treatment at 10 min holding time

Variety	Ripening days at 10°C, 95% RH	Treatment	Rheological parameters			USDA consistency (cm)	
			Consistency index (Pa.s)	n	Yield stress (Pa)	sauce	free-liquid
Cortland	0	Control	167 de	0.06 ab	147 e	3.3 abc	1.7 c
		Ca ²⁺	126 de	0.04 ab	120 e	4.7 a	1.1 cd
		PME-10	123 de	0.00 abc	125 e	3.7 ab	3.7 b
		PME+Ca ²⁺ -10	86 e	-0.01 abc	90 e	4.2 a	1.9 c
	20	Control	167 de	0.08 a	145 e	3.4 ab	0.0 d
		Ca ²⁺	179 de	0.08 a	155 e	3.4 ab	0.1 d
		PME-10	411 bc	-0.15 d	528 c	2.1 bcd	0.0 d
		PME+Ca ²⁺ -10	603 b	-0.05 bcd	680 b	1.4 cd	4.0 ab
	40	Control	127 de	0.05 ab	116 e	3.7 ab	0.3 d
		Ca ²⁺	157 de	0.07 a	136 e	3.8 ab	0.3 d
		PME-10	318 cd	-0.08 cd	405 d	2.3 bc	0.0 d
		PME+Ca ²⁺ -10	814 a	-0.03 abc	860 a	0.3 d	4.9 a
Crispin	0	Control	124 cd	0.02 ab	101 a	4.2 a	1.6 cde
		Ca ²⁺	120 cd	0.00 ab	122 e	3.2 a	2.1 bcd
		PME-10	161 cd	-0.02 abc	172 cde	3.3 b	3.1 abc
		PME+Ca ²⁺ -10	127 cd	-0.03 abc	129 e	3.7 ab	3.5 ab
	20	Control	95 d	-0.02 abc	99 e	4.2 a	1.1 de
		Ca ²⁺	96 d	0.00 ab	97 e	3.6 a	1.5 cde
		PME-10	298 ab	-0.06 abc	331 ab	2.5 b	0.0 e
		PME+Ca ²⁺ -10	375 a	-0.08 bc	447 a	2.7 ab	3.9 a
	40	Control	148 cd	0.04 a	138 de	3.4 a	1.0 de
		Ca ²⁺	153 cd	0.05 a	140 cde	3.9 a	0.9 de
		PME-10	219 bcd	-0.12 c	267 bc	2.4 b	0.0 e
		PME+Ca ²⁺ -10	239 bc	-0.04 abc	263 bcd	3.2 ab	4.0 a
Jonagold	0	Control	53 c	-0.01 ab	54 d	4.4 a	1.1 cde
		Ca ²⁺	66 c	-0.02 ab	70 d	3.9 ab	1.7 bcd
		PME-10	77 c	-0.01 ab	80 d	4.0 b	2.3 b
		PME+Ca ²⁺ -10	81 c	-0.02 abc	35 d	4.1 ab	2.1 bc
	20	Control	119 bc	0.04 a	110 d	3.9 a	1.3 bcd
		Ca ²⁺	138 bc	0.05 a	126 cd	3.5 ab	1.1 cde
		PME-10	132 bc	-0.07 bc	151 cd	3.6 b	0.1 ef
		PME+Ca ²⁺ -10	220 ab	-0.04 abc	238 bc	3.3 ab	3.8 a
	40	Control	126 bc	0.04 a	116 d	3.9 a	0.8 def
		Ca ²⁺	130 bc	0.06 a	116 d	4.0 ab	0.7 def
		PME-10	301 a	-0.12 c	372 a	2.3 b	0.0 f
		PME+Ca ²⁺ -10	284 a	-0.08 bc	344 ab	2.6 ab	3.8 a

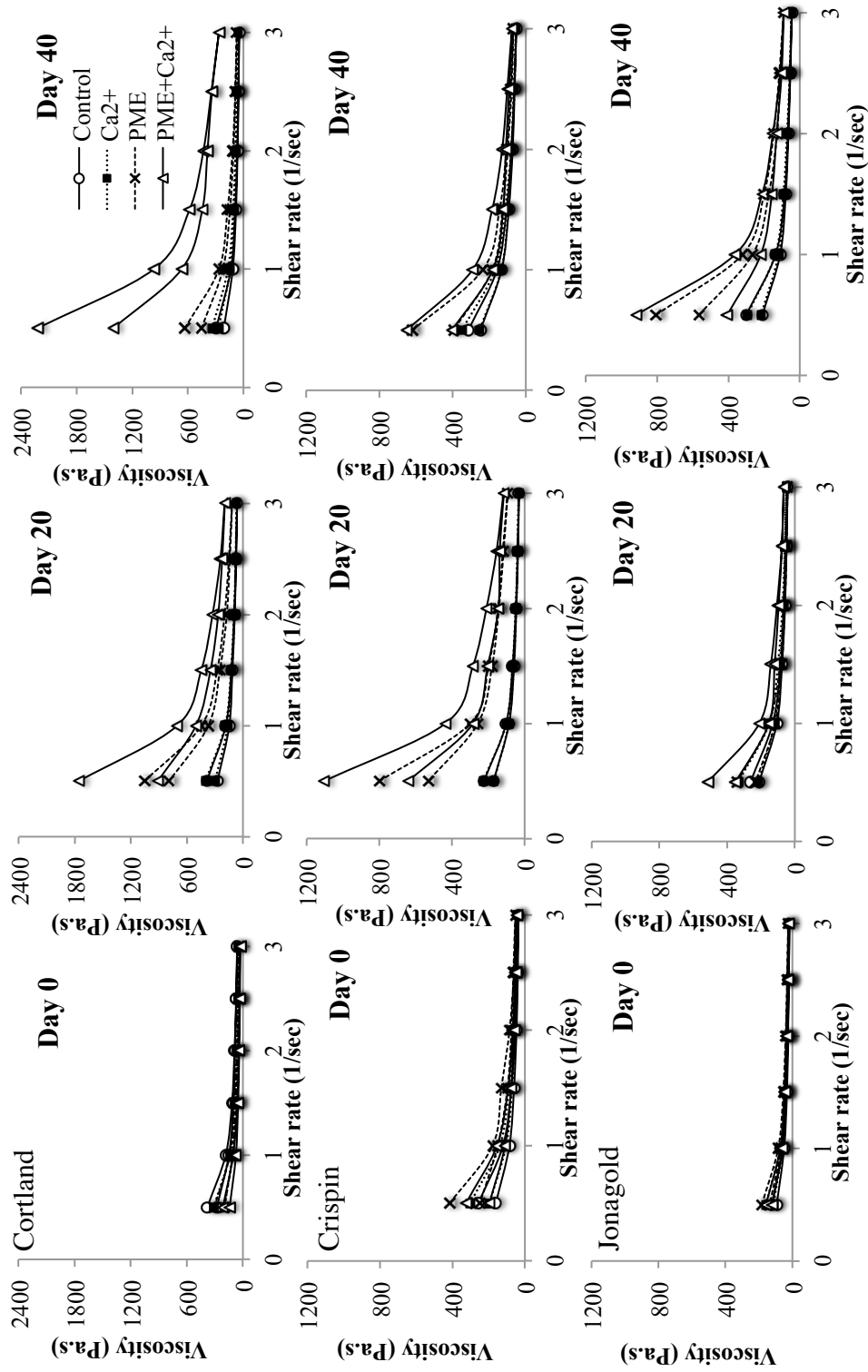


Figure 5.2 Steady-shear viscosity data of varietal applesauce made from three ripening days (ripened at 10°C, 95 % relative humidity) and obtained with the rheometer in four different treatments (Control – no treatment, Ca²⁺-200 ppm calcium solution, PME-250 ppm pectin methyl esterase, PME+Ca²⁺-250 ppm pectin methyl esterase and 200 ppm calcium solution)

from holding time (10, 20, 30 min) was observed for both PME and PME+Ca²⁺ treatment, indicating that 10 min holding time is sufficient for the enzyme to modify the pectin structure. Thus, only rheological properties of sauce processed with 10 min holding time, named PME-10 and PME+Ca²⁺-10 are presented in Table 5.1. For 20 and 40 days storage time, PME-treated sauce with and without Ca²⁺ had significantly greater consistency index and yield stress values, indicating higher viscosity than control and Ca²⁺-treated sauce. Similar observations have been reported for canned peaches (Javeri and others 1991), pasteurized strawberries (Degraeve and others 2003), apple pieces (Guillemin and others 2008), apple suspension (Bengtsson and others 2011) and parsnip suspension (Castro and others 2013). The free calcium in applesauce serum ranged from 10 to 20 ppm for control sauce, indicating that calcium was not the limiting factor for forming a stronger network. This conclusion is supported by the observation that applesauce rheology was not affected by Ca²⁺ treatment alone. According to our preliminary study, no effect of endogenous PME was found in this temperature. Consequently, the viscosity changes observed in this study could be only due to exogenous PME.

Consistency index (200-800 Pa.s) of PME+Ca²⁺-10 sauces made from apples stored for 20 and 40 days were significantly higher than previously reported for applesauce (7 – 50 Pa.s) (Rao and others 1986; Qiu and Rao 1988; Godfrey and others 1995). These uncommonly high values could indicate that sauce changed its behavior to gel-like structure with the PME treatment in the presence of added Ca²⁺ as found in tomato homogenate (Verlent and others 2006). PME hydrolyzed methyl ester groups from pectin and allowed available carboxyl groups to interact with calcium, forming

egg box structures described by Grant and others (1973). The absence of methyl esters could allow pectin chains to come closely together and increase pectin interactions, which probably contributed to the increased viscosity. Also, the reduction in negative charges of pectin polymers as a result of Ca^{2+} binding of de-methylated carboxyl groups, could help to increase particle interactions as a consequence of lowered repulsion and increased attraction between pectin chains by increasing formation of hydrogen bonds and hydrophobic interactions (Yoo and others 2009).

Table 5.1 also shows USDA consistency values for different varietal applesauces. USDA consistency sauce is negatively related to consistency index and yield stress values ($r = -0.88$ and -0.89 , respectively). For all three varieties, longer fruit storage led to lower USDA consistency sauce and free-liquid, relating to higher viscosity with less syneresis. However, when applesauce was only Ca^{2+} -treated, applesauce with a similar viscosity and free liquid separation as control sauce was obtained. For applesauce made from apples stored for 20 and 40 days, PME and $\text{PME}+\text{Ca}^{2+}$ treatment lowered USDA consistency sauce (higher viscosity), but led to different effects on USDA consistency free-liquid: the former reduced syneresis in applesauce whereas the latter increased it. The possible explanation is that since pectin molecules approach each other closely, the interaction might readily expel water from the network, resulting in more syneresis (Yoo and others 2009). In contrast, for apples processed immediately after harvest (storage time = 0 days), both PME and $\text{PME}+\text{Ca}^{2+}$ treatment resulted in slight changes in sauce viscosity and greater syneresis. As will be discussed below, changes in the pectin fraction and its DM are mainly responsible for the changes in applesauce viscosity and syneresis.

Applesauce serum of non-treated applesauce exhibited viscosities between 2-11 mPa.s, increasing with apple storage time, and decreasing to 1-4 mPa.s when sauces were subjected to PME activity. The decrease in serum viscosity with PME treatment could be explained by the change in pectic substances from soluble to insoluble materials, which are in agreement with previous studies by Osorio and others (2008) and Castro and others (2013).

Influence of exogenous PME on alcohol insoluble residue (AIR), galacturonic acid (GalA) content and pectin degree of methoxylation (DM)

AIR, representing the sum of soluble and insoluble fiber, varied from 2.00% to 3.60% as shown in Table 5.2, and the range of results was comparable to those found in the literature for apples and applesauce (1.76-5.48%) (Fischer and others 1994; Colin-Henrion and others 2009). AIR significantly decreased with apple storage time for all three varieties; however, no effect of PME treatment was found, showing no modification on total fiber content. Due to differences in the solubility of pectic polymers, AIR was fractionated to obtain water-soluble pectin (WSP) and chelator-soluble pectin (CSP).

The GalA content and DM of WSP and CSP varied significantly as a result of apple storage time and PME treatment ($p < 0.05$) as shown in Table 5.2. GalA content and DM of pectin obtained from the non-treated sauce ranged from 0.2 to 0.5% and 55 to 80%, respectively, corresponding well to previous studies (Johnston and others 2002; Scalzo and others 2005; Le Bourvellec and others 2011). During ripening, GalA

Table 5.2 Galacturonic acid (Gala) content (g Gala/100g applesauce) and degree of methoxylation (%) (average values \pm standard deviation of the mean) of water-soluble pectin (WSP) and chelator-soluble pectin (CSP) extracted from applesauce treated with exogenous pectin methyl esterase (PME) at 10 min holding time

Variety	Ripening days at 10°C, 95% RH	Treatment	Alcohol insoluble residue (%)	Galacturonic acid content (%)		Degree of methoxylation (%)	
				WSP	CSP	WSP	CSP
Cortland	0	Control	3.51 \pm 0.16 a	0.21 \pm 0.03 a	0.02 \pm 0.00 b	60 \pm 8 b	39 \pm 2 b
		PME-10	3.34 \pm 0.43 a	0.06 \pm 0.01 b	0.05 \pm 0.01 a	30 \pm 3 c	31 \pm 1 c
	20	Control	2.58 \pm 0.02 b	0.25 \pm 0.04 a	0.04 \pm 0.01 b	61 \pm 4 b	44 \pm 7 b
		PME-10	2.35 \pm 0.12 b	0.17 \pm 0.04 b	0.09 \pm 0.01 a	14 \pm 1 cd	12 \pm 0 c
	40	Control	2.10 \pm 0.04 c	0.26 \pm 0.05 a	0.06 \pm 0.02 b	61 \pm 6 b	45 \pm 2 b
		PME-10	2.07 \pm 0.06 c	0.21 \pm 0.08 b	0.07 \pm 0.04 a	8 \pm 3 d	12 \pm 8 c
Crispin	0	Control	3.60 \pm 0.55 a	0.14 \pm 0.00 b	0.07 \pm 0.00 a	75 \pm 3 ab	71 \pm 1 a
		PME-10	3.56 \pm 0.72 a	0.26 \pm 0.11 a	0.15 \pm 0.01 a	4 \pm 1 d	8 \pm 5 c
	20	Control	2.65 \pm 0.13 b	0.20 \pm 0.01 b	0.08 \pm 0.02 a	87 \pm 0 a	71 \pm 1 a
		PME-10	2.36 \pm 0.24 b	0.22 \pm 0.02 a	0.12 \pm 0.11 a	5 \pm 0 d	8 \pm 5 c
	40	Control	2.41 \pm 0.08 c	0.17 \pm 0.02 b	0.08 \pm 0.00 a	79 \pm 6 ab	67 \pm 0 a
		PME-10	2.41 \pm 0.03 c	0.25 \pm 0.06 a	0.12 \pm 0.04 a	6 \pm 2 d	5 \pm 2 c
Jonagold	0	Control	2.95 \pm 0.23 a	0.23 \pm 0.01 ab	0.06 \pm 0.01 bc	67 \pm 7 b	58 \pm 6 a
		PME-10	3.06 \pm 0.04 a	0.10 \pm 0.00 b	0.15 \pm 0.04 a	19 \pm 5 cd	12 \pm 2 c
	20	Control	2.28 \pm 0.08 b	0.34 \pm 0.09 a	0.11 \pm 0.00 ab	71 \pm 4 ab	75 \pm 10 a
		PME-10	2.14 \pm 0.24 b	0.08 \pm 0.03 b	0.06 \pm 0.02 bc	20 \pm 7 cd	15 \pm 2 c
	40	Control	2.17 \pm 0.02 c	0.14 \pm 0.02 b	0.03 \pm 0.01 c	74 \pm 9 ab	53 \pm 4 a
		PME-10	2.03 \pm 0.01 c	0.14 \pm 0.06 b	0.10 \pm 0.03 abc	9 \pm 4 d	3 \pm 1 c

Different letters in the same column within the same variety indicate significant differences at the 0.05 level (Tukey's HSD test)

content of WSP and CSP increased from apple storage time 0 to 20 days due to pectin depolymerization, converting protopectin to soluble pectin and then decreased from apple storage time 20 to 40 days due to pectin degradation (Billy and others 2008), but DM of WSP and CSP remained similar over apple storage.

DM of WSP and CSP differed considerably between the different varieties: Crispin > Jonagold > Cortland and was significantly affected by PME treatment ($p < 0.001$). The DM decreased from 40-87% to 3-30% with PME treatment, and WSP was generally more highly esterified than CSP (Table 5.2). Lowering DM by exogenous PME caused a decrease in GalA content in WSP, which was accompanied by an increase in CSP, except for Crispin where GalA content in WSP increased with PME treatment. The higher amount of GalA content in CSP could be attributed to the demethoxylation of pectin as the increase in blocks of unesterified GalA residues provides a greater opportunity for the pectic polymers to be cross-linked with divalent ions such as Ca^{2+} , generating a high amount of CSP fraction. Similar results were reported by Lisiewska and others (2006), Christiaens and others (2012a and 2012b) and Castro and others (2013) showing that WSP fraction increased when CSP fraction decreased in fruit purees or suspension subjected to PME activity.

Influence of exogenous PME on particle size distributions

The particle size distribution was unimodal for most applesauces, except for control sauce at day 0, showing bimodal distribution, as can be seen in Figure 5.3. At day 0, the small peak representing small particles (4-70 μm) disappeared when the sauce was treated with PME, with the increased volume of large particles. The

volume-weighted mean diameter (d_{43}) was significantly different among varieties: Jonagold > Cortland > Crispin, but responded to PME treatments similarly. The effect of PME treatment on d_{43} of sauce was dependent on apple storage time; causing it to increase, having no effect, and causing it to decrease at apple storage time 0, 20, and 40; respectively. No effect from calcium addition was observed. Previous studies have reported that mean particle diameter increased with PME treatment in orange juice and pectin gel due to the formation of non-methoxy ester linkages between pectin molecules (Corredig and Wicker 2002; Wang and others 2013); however, other studies found no PME effect on mean particle diameter in parsnip suspension (Castro and others 2013). Consequently, the effect of PME treatment on particle size depends on other factors such as type of fruits and post-harvest fruit ripening stage. The decrease in d_{43} resulted in increasing consistency index and yield stress ($r = 0.41$ and 0.42 , respectively) and decreasing USDA consistency free-liquid ($r = 0.33$). This had been ascribed to an increase in the number of interactions between particles or to the fact that smaller particles fit better in the pectin network (Beresovsky and others 1995), forming stronger a network and better water binding capacity.

Activation of endogenous PME and its effect on applesauce properties

The amount of AIR, GalA content and DM in both WSP and CSP are shown in Table 5.3. The DM of pectin extracted from control sauce (no holding) was estimated at 83% for WSP and 45% for CSP, which was in agreement with the value previously found in the exogenous PME study for Crispin and Jonagold. Holding applesauce at 55-60°C significantly induced a decrease in the DM of WSP to 63-70% but DM of

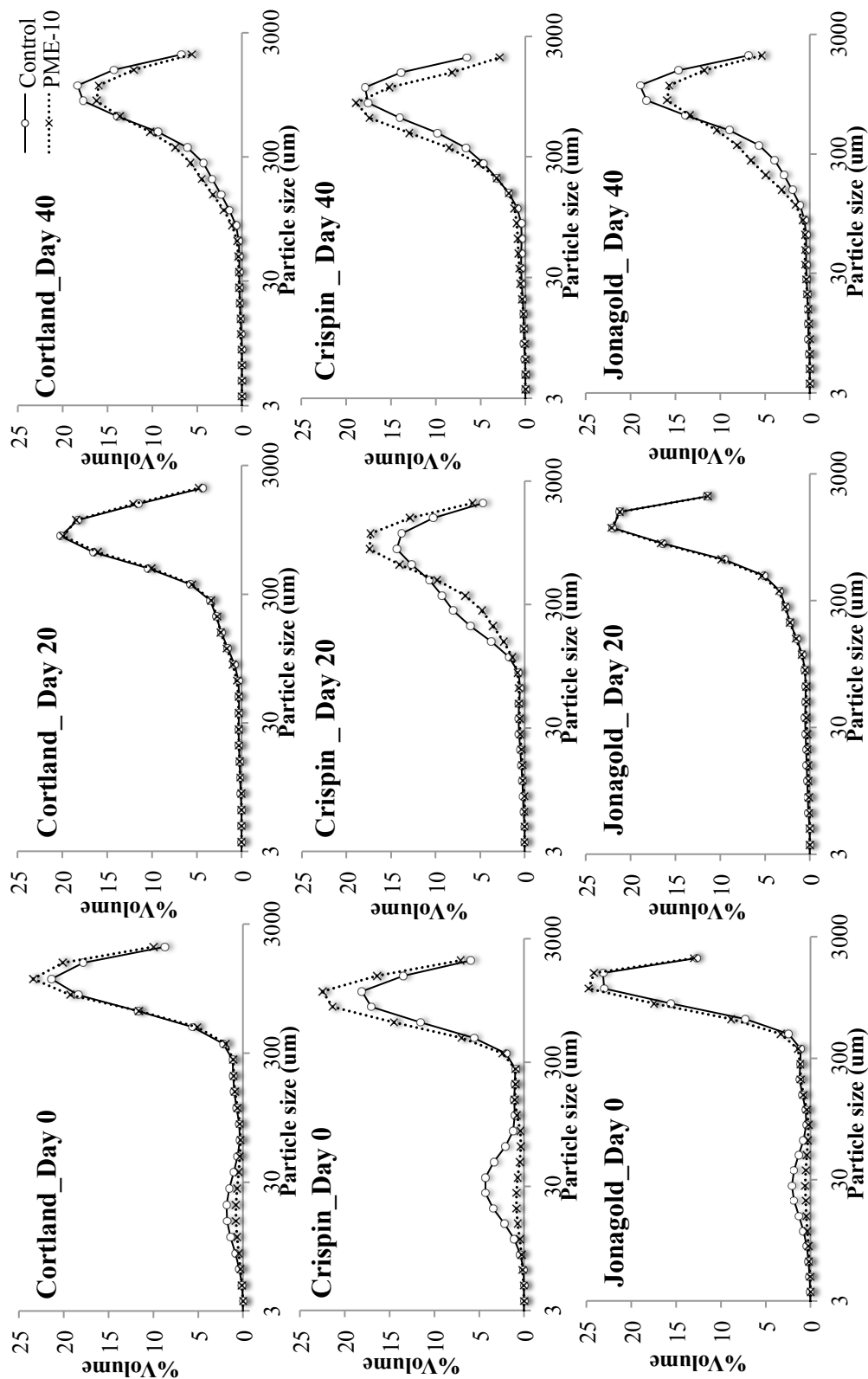


Figure 5.3 Particle size distribution of varietal applesauce made from three ripening days (ripened at 10°C, 95 % relative humidity) (Control – no treatment, PME-10 – 250 ppm pectin methyl ester with 10 min holding time)

CSP did not change. When holding at higher temperature (65-71°C), DM of both WSP and CSP was not significantly different from control. Additionally, there was no significant difference in pectin DM when holding at 60°C for 5, 10 and 30 min. Therefore, the optimal condition to activate endogenous PME in applesauce was 55-60°C, which was in agreement with previous reports that the endogenous PME activity was enhanced during low-temperature blanching, typically 15 to 45 min at 50 to 60°C (Ni and others 2005; Sila and others 2005). Endogenous PME did not affect AIR and GalA content of WSP and CSP. However, a lower amount of GalA in CSP was found when holding at 55°C, indicating pectin degradation caused by pectinase activity, and less change was observed at higher temperatures due to enzyme inactivation.

Despite the lower DM of pectin, no significant differences were observed between control and treated sauce on rheological parameters (consistency index, n , yield stress and USDA consistency values) and particle size distribution. The main difference between exogenous and endogenous PME activity was that the first reacted with both WSP and CSP, but the latter reacted with only WSP. Van Buggenhout (2009) reported that fungal PME is more active in acidic conditions than plant PME; therefore, DM of pectin was lower when treated with exogenous PME. Unlike plant PME, the fungal PME activity resulted in a random de-esterification pattern, which has a positive effect on the strength of the formed gel (Lofgren and others 2005), which helps explain the different effects of PME activity on sauce rheology found on our studies.

Table 5.3 Changes in alcohol insoluble residue, galacturonic acid (GalA) content and degree of methoxylation (average values \pm standard deviation) of water-soluble pectin (WSP) and chelator-soluble pectin (CSP) in applesauce due to activation of endogenous pectin methyl esterase

Holding temperature (°C)	Holding time (min)	Alcohol insoluble residue (%)	GalA content (g GalA/100g applesauce)		Degree of methoxylation (%)	
			WSP	CSP	WSP	CSP
Control	-	1.89 \pm 0.07	0.99 \pm 0.09	0.73 \pm 0.14 a	83 \pm 3 a	45 \pm 3
	10	1.74 \pm 0.04	1.00 \pm 0.20	0.50 \pm 0.09 b	65 \pm 3 cd	37 \pm 9
60	5	1.80 \pm 0.06	0.88 \pm 0.18	0.57 \pm 0.06 ab	62 \pm 4 d	42 \pm 3
	10	1.83 \pm 0.03	0.91 \pm 0.14	0.67 \pm 0.06 ab	64 \pm 4 cd	40 \pm 6
	30	1.74 \pm 0.13	1.01 \pm 0.15	0.51 \pm 0.09 ab	70 \pm 6 bcd	40 \pm 6
65	10	1.76 \pm 0.10	0.93 \pm 0.08	0.64 \pm 0.03 ab	75 \pm 3 abc	37 \pm 5
	10	1.89 \pm 0.12	0.84 \pm 0.04	0.63 \pm 0.02 ab	77 \pm 3 ab	41 \pm 2

Different letters in the same column indicate significant differences at the 0.05 level (Tukey's HSD test)

Conclusion

Pectin DM is an important factor that affects applesauce viscosity and could be decreased by both exogenous and endogenous PME activity. Exogenous PME addition induced higher viscosity and less syneresis for applesauce processed from cold stored apples by lowering the DM for both water soluble and chelator soluble pectin from 40-87% to 3-30%. However, the highest syneresis was found when applesauce was treated with both PME and Ca^{2+} . Endogenous PME had no impact on sauce viscosity even though it was activated by holding sauce at 55-60°C for 10 min, resulting in a decrease of DM in water soluble pectin from 83% to 63-70%. Therefore, to have a significant impact on sauce rheology, a change in pectin DM of more than 20% would be required.

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CHAPTER 6

CONCLUSION AND FUTURE WORK

Apple variety and postharvest ripening have a large influence on applesauce quality. Applesauce made from ripened apples normally has better quality (thicker sauce with less syneresis) than one made from fresh apples. Applesauce consistency is related to physical and chemical attributes, most importantly mean particle diameter, particle size distribution span, total soluble pectin content, and pectin degree of methoxylation. Processing operations also impact sauce quality as changes in specific parameters can result in targeted attributes.

Our study determined the difference between hot and cold break process with three extractor rotational speeds on rheological properties of varietal applesauce processed at different ripening stages. Results indicated that hot break sauce had significantly higher total soluble pectin content, smaller mean particle diameter, wider particle size distribution, and less syneresis than cold break sauce processed from the same variety and ripening stage. Sauce viscosity however depended on ripening stage – the cold break process produced thicker sauce when processed from ripened apples. Also, increasing extractor rotational speed, for both hot and cold break process, resulted in higher applesauce consistency and decreased free liquid separation. Additionally, hot break sauce produced consistent sauce quality with less syneresis, regardless of variety and ripening; however, the hot break process required longer processing time and higher production cost due to the steam consumption. Therefore, combining both hot and cold break processes could optimize product quality and

operation cost.

We studied the effect of addition of diced apples (three different dice sizes and four proportions) representing hot break process, to the extractor sauce output from cold break process before final pulping or finishing, on varietal applesauce rheology. Regardless of dice size and variety, the addition of diced apples into the cold break output resulted in thicker sauce, less syneresis, higher total soluble pectin content, smaller mean particle diameter, and wider particle size distribution. Sauces made with at least 50% cooked, diced apple addition showed significant sauce quality improvement for all three varieties, and the effects were more pronounced in challenging varieties, the ones typically making thin sauce. Thus, applesauce processors can use this finding to overcome the variability associated with apple variety and ripening stage to achieve consistent, good sauce quality all year round.

In the final study, we evaluated the effect of exogenous pectin methyl esterase (PME) addition on consistency of cold break sauce and the activation of endogenous PME to assess its effect on consistency of the mixture of diced apple (hot break) and extractor sauce output (cold break). Exogenous PME addition caused a decrease in degree of methoxylation of water- and chelator-soluble pectin from initial levels of 40-87% to 3-30%, leading to substantially higher sauce viscosity and less syneresis. When combined with Ca^{2+} , added as calcium chloride, applesauce had the highest viscosity but also the greatest syneresis. The optimal condition to activate endogenous PME was holding the mixture of extractor output and diced apples at 55-60°C for 10 min, leading to a decrease of degree of methoxylation of water soluble pectin from 83% to 60-70%. However, no change on applesauce consistency was observed. Even

though exogenous PME can improve sauce quality, the addition of it into applesauce is not currently allowed based on 21 CFR 145.110.

Based on the results from this research, it can be concluded that applesauce consistency depends on its physical and chemical attributes, which are influenced by variety and post-harvest storage time, and altered by processing treatments. Based on our findings, the recommended processing conditions for the achievement of good applesauce quality are to add at least 50% of diced apples to the cold break sauce output before pulping to final products, and immediately heat up to 90°C to pasteurize and deactivate enzymes without any holding condition. Good sauce quality (USDA consistency sauce ≤ 6.5 cm and USDA consistency free-liquid flow ≤ 0.7 cm) should have mean particle size 500-700 μm , particle size distribution span 1.75-2, total soluble pectin 0.25-0.4 %, and pectin degree of methoxylation 40-60 %.

Our results provide a scientific basis to explain how processing conditions affect the rheological, physical, and chemical properties of applesauce, which can be used by the processing industry to improve applesauce quality and similar products.

With this research, we are able to show that applesauce, made from different apple varieties and ripening stages, is affected differently by processes such as heat treatment as evidenced by changes in the chemical composition and particle size distribution, which, in turn, affect applesauce consistency. Thus, it would be very important to study more apple varieties and ripening stages to provide global knowledge for applesauce processors, allowing the prediction of final product based on initial attributes of apples. Processing treatments such as heating could be used to improve product quality when needed, which could help manufacturers avoid

producing substandard quality sauce, leading to reduced operation costs as reprocessing and waste are minimized. Energy consumption can be optimized since the hot break process will be used only when required such as to process applesauce from challenging varieties and/or freshly harvested apples.

Sugar addition to applesauce, a common practice to manufacture sweetened sauce, affects applesauce consistency and therefore, it would be of interest to determine which varieties and ripening stages are appropriate to produce sweetened applesauce and whether the processing conditions can be used to improve sauce quality as found in unsweetened applesauce.

Additionally, pectin is found to be the most important component as a gelling or binding agent, contributing to applesauce consistency; therefore, it would be of much value to further investigate the relationship to pectin structure. Recently the use of anti-homogalacturonan antibodies, which have binding capacity to specific pectin components, has been introduced as a tool to characterize pectin composition. This technique can define degrees and patterns of methyl-esterification found in the pectin structure. Therefore, combining this approach with data of pectin content and degree of methoxylation can provide a more in-depth view of the pectin structure-function relationship and process-induced changes in pectin structure, which will be applicable to applesauce and other fruit purees or sauces.

This work has also provided further insight for future studies related to potential applications of processing treatments for improving product texture in other fruits. For example, thermal treatment of diced apple prior to pulping showed an increase in sauce water-binding capacity. Thus, It would be interesting to investigate

whether the thermal pretreatment can be applied to all types of fruit purees and sauces such as tomato, strawberry, papaya, mango, and apricot. Moreover, relationships were found between the product consistency, the chemical composition of the pectin and the particle size distribution. Other fruit and vegetable purees and sauces should be studied to verify this relationship.